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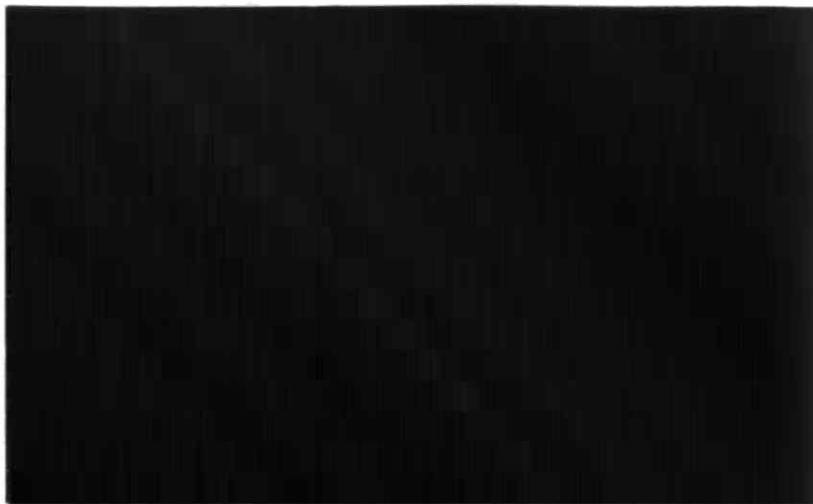
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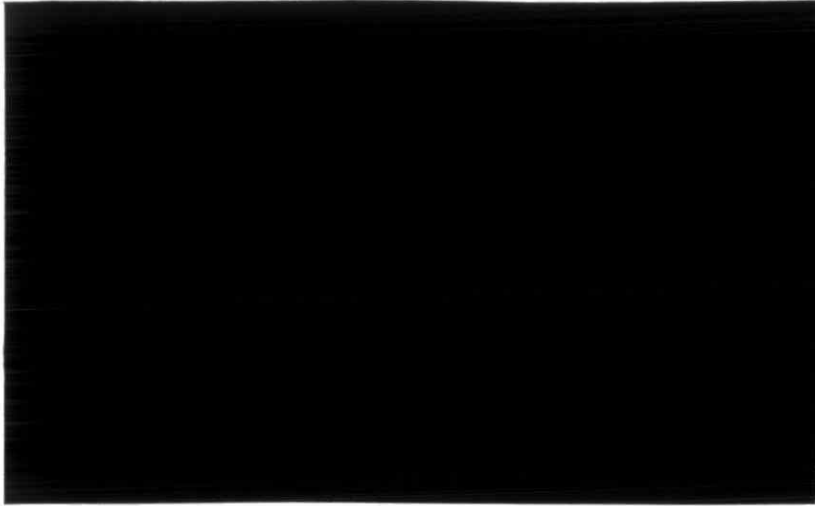
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IN SITU ASSESSMENT OF
COPPER AND ZINC IMPACTS
ON WHITE SUCKER POPULATIONS
OF THE MANITOUWADGE CHAIN
OF LAKES

R. A. C. PROJECT NO. 193 RR

Report prepared for Environment Ontario by:

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1988

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ABSTRACT

This project was undertaken as an integrated field-laboratory program designed to determine the impacts of copper and zinc contamination on white sucker (Catostomus commersoni) populations in several lakes in the Manitouwadge district of Northern Ontario. White suckers were collected from lakes with elevated levels of both copper ($13-15 \mu\text{g l}^{-1}$) and zinc ($209-253 \mu\text{g l}^{-1}$) over the period of 1985-1987. Suckers were evaluated for evidence of impact on growth, reproductive performance, larval survival and tolerance of larvae to copper exposure.

After the age of sexual maturation, contaminated fish were significantly smaller and shorter, and female suckers failed to exhibit significant increases in either length or weight with age. The fish from contaminated lakes also exhibited decreases in egg size and fecundity, and exhibited an increased incidence of spawning failure. Alterations in sucker growth, lipid reserves and fecundity appear to be related to nutritional deficiencies as a result of the chronic effects of elevated sediment metals on the food base of the suckers.

Examination of the reproductive performance did not detect differences in suckers from contaminated sites. However, larvae hatched from eggs collected at contaminated sites exhibited differences in size, developmental rate, survival and growth, relative to controls. Larvae from contaminated eggs showed increased resistance and tolerance to water-borne copper during the period of endogenous nutrition. This study provides evidence for a maternal yolk factor associated with increased resistance and tolerance of larvae to copper. Incubation of eggs in streams flowing out of the tailings area resulted in a decreased egg size and tolerance to copper.

In addition to the field validation, a framework was developed as a simple, cost-effective, rapid mechanism for assessment of toxicant impact on aquatic environments. The framework is known as Population Indicators of Sublethal Contaminant Effects on Suckers (PISCES), and separates response patterns based on population characteristics. The application of PISCES to this study, and to several published data sets, showed that white sucker populations responded to environmental stressors in a predictable fashion. Limitations of PISCES and requirements for the further development of a model for field validation and environmental health assessment are discussed.

EXECUTIVE SUMMARY

The project was undertaken as an integrated field-laboratory program designed to determine the impacts of copper and zinc contamination on white sucker (Catostomus commersoni) populations in several lakes in the Manitouwadge district of Northern Ontario. White sucker were collected from lakes containing elevated levels of both copper (13-15 ug/l) and zinc (209-253 ug/l) over the period of 1985-1987. Suckers were evaluated for evidence of impact on growth, reproductive performance, larval survival and tolerance of larvae to copper and zinc exposure.

In all cases, sucker reached the age of maturity between 4 and 6 years of age, and until 6 years of age, there were no differences in length or weight of fish from control and contaminated sites. After this age, fish from contaminated sites were significantly smaller and shorter than those from control sites. In addition, fish from contaminated lakes also exhibited decreases in fecundity and egg size, failed to show significant increases of fecundity with age and exhibited an increased incidence of spawning failure.

The failure of the female fish to grow significantly after maturity, and the decreased energetic commitment to reproduction suggests that the food base in the contaminated lakes was limiting the performance of the female sucker. These fish exhibited decreased muscle lipid levels, decreased serum lipid levels during the post-spawning period and an apparent decrease in visceral lipids during the autumn. There was no effect of collection site on body stores of liver glycogen, liver lipids, serum triglycerides or total serum cholesterol.

Examination of the reproductive performance did not detect differences in sucker at contaminated sites. Fertilization success was not impaired in white sucker from contaminated sites; there were no differences detected in naturally-fertilized eggs at contaminated sites and metal-exposed males performed better than control males in fertilization trials with control eggs.

Larvae hatched from eggs collected at contaminated sites were smaller, developed at a slightly increased rate, and exhibited poorer growth and survival than larvae from control sites. These changes were evident despite the fact that the contaminated eggs were fertilized and hatched in clean water, and the differences are consistent with both the phenomena of decreased female energetic commitment and vertical transmission of contaminant residues.

Larvae showed significant changes in tolerance and resistance to copper and zinc with age. Copper resistance peaked at the time of the onset of liver functioning, and larvae from contaminated eggs showed increased resistance and tolerance to water-borne copper during the periods of endogenous nutrition, despite the fact that the eggs were not pre-exposed to exogenous metals. The effect was not seen in larvae at first feeding, at ages older than 4 d after the onset of feeding or in larvae hatched from control eggs fertilized with sperm taken from males at contaminated sites. This study provides evidence for a maternal yolk factor associated with increased resistance and tolerance of larvae to copper. The factor appears to be metal residues transferred in the yolk, and no differences were detected in egg metallothionein residues between control and contaminated sites.

Naturally-fertilized eggs were collected from the spawning sites, and eggs collected at contaminated sites exhibited a further decrease in egg size and increased

deformity rate not evident in contaminated eggs manually fertilized in control water. Incubation trials involving the placement of eggs in streams flowing out of the tailings area resulted in a decreased egg size and tolerance to copper and an increased deformity rate. Both changes appear to result from the influx of metals during the water-hardening process.

The distribution of metals in sucker tissues was monitored, and elevations in both copper and zinc residues were identified in liver, kidney, gill and gonadal tissue. There was evidence of metal uptake from the diet and the concentrations of metals in gut contents exceeded 400 ppm Cu and 1200 ppm Zn. Analysis of sediment metal concentrations showed elevations in both Cu and Zn at contaminated sites.

Additional work shows that several major food groups are missing from the sediments of contaminated sites, and previous work suggests that sediments under water deeper than 5 m may be incapable of supporting macroinvertebrate fauna. Analysis of benthic samples indicated a decreased abundance or absence of pollution-sensitive groups such as ephemeroptera, plecoptera, odonata, hirudinea, unionid clams, gastropods, amphipods and aquatic beetles. Fauna at the contaminated sites was dominated by chironomids and other dipteran species.

Effects on the growth and fecundity of sucker were attributed to nutritional deficiencies related to the decreased food abundance and density at contaminated sites, which could be related to the increased sediment metal levels. Sediment metal burdens have declined substantially since the late 1960's. Direct effects of the metals were detected on the larvae hatching from eggs collected at contaminated sites. Evidence for direct effects on egg size and larval deformities was related to increased

metal burdens in the eggs. This increase could be related to both the entry of metals during the water-hardening process at contaminated sites and the vertical transmission of metal residues from the female through the yolk.

In addition to field validation, a framework was developed as a simple, cost-effective, rapid mechanism for assessment of toxicant impact on aquatic environments. The framework, Population Indicators of Sublethal Contaminant Effects on Suckers (PISCES), separated response patterns based on population characteristics. The framework assumes that changes in the death or birth rates of fish populations, or alterations in the availability of food or habitat are associated with characteristic responses of sucker populations. The responses have been grouped into five main patterns based on the population characteristics of mean age, fecundity and condition factor. Populations which are growing, reproducing or surviving at rates which are indistinguishable from a reference (control) population are considered free from adverse chemical effects.

The application of PISCES to this study, and to several previously published data sets, showed that white sucker populations responded to environmental stressors in a predictable pattern. Limitations of PISCES and requirements for the further development of a model for field validation and environmental health assessment are discussed.

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Chapter I

GENERAL INTRODUCTION

1.1 Background

The major approach to defining the impact of toxic chemicals in the aquatic environment has, to date, been the derivation of water quality objectives using laboratory-based toxicity testing. Current water quality objectives have been developed using a toxic threshold or "no-effect level" and an arbitrary application factor (Barnthouse et al., 1987). Such objectives usually take into account the effects of dominant modifying factors, although such data is currently absent for many contaminants. The water quality objectives are based on data for sensitive species, calculated from well-controlled experiments and provide a relatively inexpensive approach to water quality management. Although this approach has many limitations, it is used throughout North America because of its assumed predictive capabilities.

Unfortunately, the water quality objectives approach is based on the untested and probably false assumption that an environment is protected if the objectives are met (NRCC, 1985). While considerable resources have been spent on classical aquatic toxicology, the degree of contaminant impact on free-living organisms has rarely been defined, nor are there widely-accepted, reliable methods for determining the extent of these impacts (Gilbertson, 1984; NRCC, 1985). Much recent attention has been focused on the identification and quantification of sublethal responses which

can be monitored in free-living organisms. Attempts have been made to integrate field and laboratory studies on the impact of mercury (Saikka et al., 1978; Skei, 1978), mixed metals (McFarlane and Franzin 1978; Roch et al. 1982; Klaverkamp et al., 1984; Larsson et al. 1985; Hall et al. 1987), organochlorines (Meyrle et al., 1982) and mixed contaminants (Pearce, 1984; Chapman et al. 1985). The outcomes of field-laboratory comparisons are often variable, and can be dependent on laboratory conditions and/or toxicant-specific whole organism responses (Boyle, 1985; Suter et al., 1985, 1987).

Extrapolations from the laboratory to the field are complicated by the cyclical, fluctuating nature of environmental factors and the poor understanding of the mechanisms regulating population size (Levin, 1982; Moore and Ramamoorthy, 1984). Furthermore, much of the testing involves the examination of biochemical parameters. Although this approach is known to be valuable in situations where the contaminants are well-defined (Hodson et al., 1984; Klaverkamp et al. 1984), their use in situations involving toxicant mixtures or unknown contaminants may require the development of generalized biochemical tests (Larsson et al., 1985; NRCC, 1985). Such tests are of limited value at present due to the stresses associated with capture, inadequate correlations between biochemical and whole animal effects (Neff, 1985) and the requirement for the standardization of field tests with respect to biotic modifying factors (Larsson et al., 1985; NRCC, 1985).

There are a large number of environmental and cultural stressors which can have a profound effect on fish populations. Examinations of Great Lakes fish stocks have identified major stressors such as overfishing, the introduction of exotic species, eutrophication, stream alterations, wetland depletion, sediment extraction, boating

disruptions, the loss of natural littoral zone associated with dredging, dumping or sedimentation and stressors associated with electrical power generation and toxic contamination (Johnson and Bergman, 1984; Rapport, 1984; Ryder and Edwards, 1985).

Under field conditions, a stressor will rarely be selectively lethal to any single component of an ecosystem and will usually elicit sublethal effects on other species (Wedemeyer et al., 1984). The resiliency of an ecosystem may be defined by its ability to maintain its community structure under the influence of stressors (Ryder et al., 1981; Ryder and Edwards, 1985). A comparison of differences between species in similar ecosystems should provide clues to the limits of variations and responses, regardless of the identity of the stressor (Colby and Nepszy, 1981). Such characteristic changes have been defined (Colby, 1984; Rapport, 1984; Ryder and Edwards, 1985), but changes are not always evident, and variations at lower concentrations are dependent upon environmental parameters (Moore and Ramamoorthy, 1984) and acclimatory responses (Sprague et al., 1984).

1.2 Statement of the Problem

Large scale water and sediment monitoring programs are invaluable for the qualitative identification of potentially sensitive areas, but are limited in their ability to quantitatively assess biological effects within any particular system. The enormous amount of interlake variation in modifying factors, physical characteristics, additional stressors and species composition require that potential problem areas be evaluated on an individual basis, once they have been identified. At present, there is not any generally-accepted plan for the systematic identification of ecosystems or areas where

rehabilitation is desirable, and there are not procedures to follow improvements as they occur (Cairns, 1981). The Great Lakes Fisheries Commission has identified the need for a protocol for the integration of field and laboratory studies and the establishment of correlations between changes in the laboratory and the field (Johnson and Bergman, 1984; Ryder and Edwards, 1985). Such a procedure would be essential for evaluation of damage and for the development of site-specific water quality objectives. If biological monitoring is to be successful on a large scale, then new and rapid, inexpensive methods will be required to quantitatively assess ecosystem impact in situ. The need for testing methodology has led to a number of recent conferences and workshops dealing with ecosystem health assessment (McIntyre and Pearce, 1980; Cairns et al., 1984; Levin and Kimball, 1984). Under this testing philosophy, changes evident in the organism are related to a summation of environmental stressors, including contaminants, which impinge upon them. Any assessment program would have to meet several requirements: it should be rapid and inexpensive, able to detect meaningful biological impact, and have few requirements in the field with respect to collection of samples, processing or storage.

1.3 Objectives and Organization of the Thesis

The original objectives of the study were to

1. examine the fish populations in a contaminated area for evidence of impact of the contaminants
2. examine the tolerance of larvae for evidence of compensatory adjustments to the chronic field exposure of toxicants

3. develop a protocol for the systematic identification of contaminant impact on aquatic environments

This thesis contains five main chapters, a chapter introducing the study site, rationale and scope of the study, and four chapters containing the bulk of the data. The chapters are:

1. Growth and Reproduction: most of the data collected on adult fish and the laboratory follow-up studies on the physiology of the fish.
2. Larval Survival, Growth and Tolerance to Copper: the data from the first group of eggs tested. The chapter includes both field and laboratory observations of egg development and survival, as well as larval response to challenges with copper in the laboratory.
3. Effects of Stream Incubation on Larvae: data on the follow-up egg studies. These studies were designed to detect the effects of stream incubation in contaminated water on subsequent egg development and larval response to copper and zinc exposures. Additional work was concerned with investigating the phenomena associated with altered larval responses to copper exposure in the lab, and the effects of temperature on larval development.
4. PISCES Framework: the development of a cost-effective framework for preliminary, rapid assessment of ecosystem quality and application of the framework to other published studies to test its utility.

The relative value of complete population monitoring and the PISCES framework are presented in the General Discussion along with interrelationships of the various chapters. Also included in the discussion are the limitations of PISCES and requirements for its future development.

Chapter II

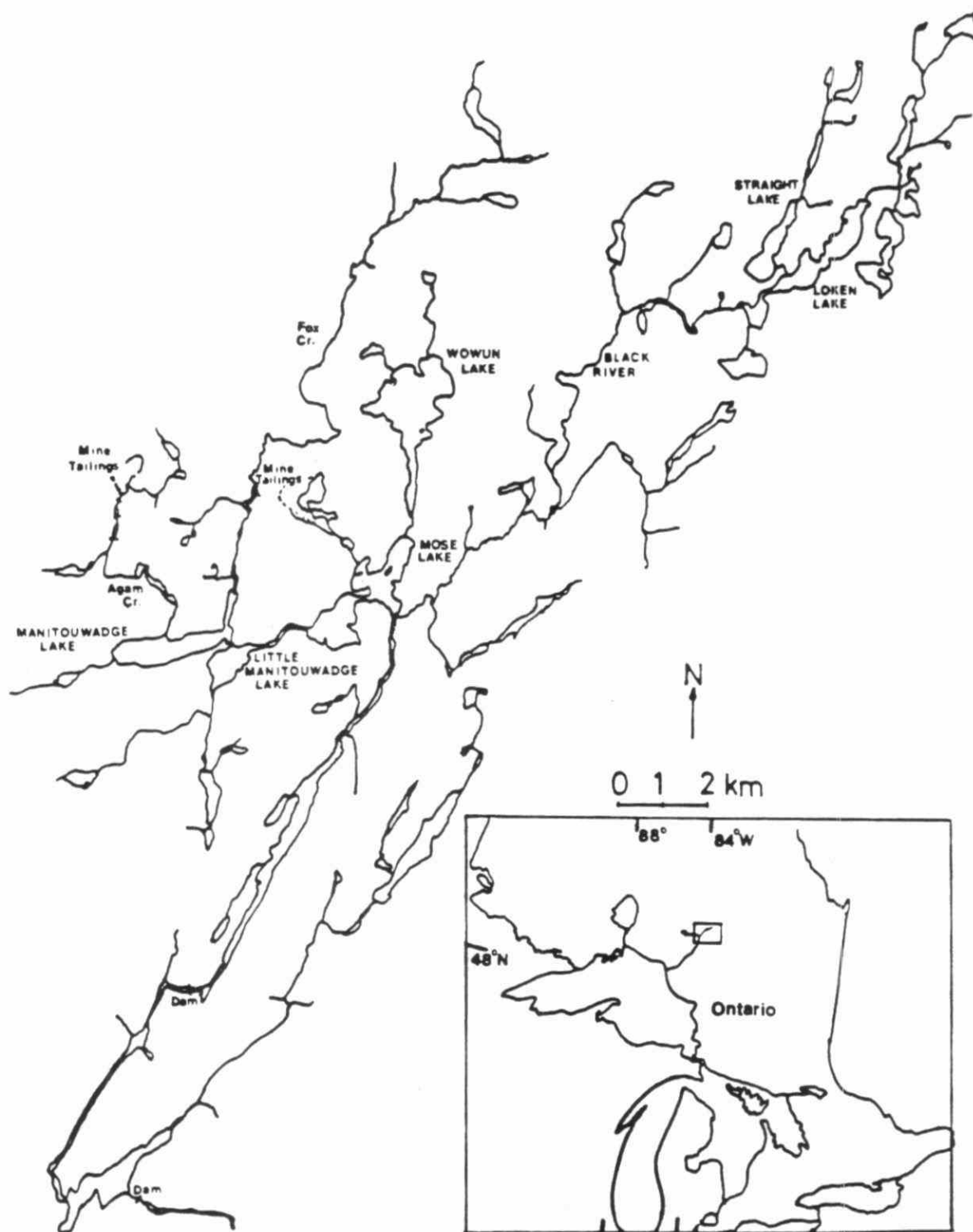
STUDY SITE, SENTINEL SELECTION AND LEVEL OF SURVEILLANCE

2.1 Study Area and Site Description

The Manitouwadge chain consists of a number of small lakes (80 to 320 ha) near the headwaters of the Black River in Northern Ontario (approximately 49°N, 85°45'W) where copper, zinc and silver have been actively mined since 1957 (Figure 1). Contaminated runoff reaches the chain through both Manitouwadge and Mose Lakes, and the Ontario Ministry of the Environment has been actively monitoring the water levels of copper and zinc for a number of years. The lakes are of intermediate hardness (108 to 112 mg l⁻¹ as CaCO₃), neutral pH (6.9-7.1) and have historically shown increased levels of copper (16 ug l⁻¹) and zinc (340 to 450 ug l⁻¹) (Hollinger, pers. comm.).

Nine lakes from the Manitouwadge chain were evaluated on the basis of accessibility, preliminary sample collections and historic water quality. All the lakes were similar in terms of mean depth and surface area. In addition, spawning areas were evaluated on the basis of accessibility, size of spawning run, natural obstructions and possible hazards to sample collection. On the basis of all criteria, Manitouwadge Lake (MAN) was selected as the elevated metal site, Little Manitouwadge Lake (LMN) was selected as the intermediate lake and Loken Lake (LOK) was selected as the control site. A small number of additional samples were collected from Mose

Figure 1: Map of the study site. Waste enters the chain through both Manitouwadge and Mose Lakes, while control sites were represented by Loken, Wowun and Straight Lakes. Water flow is toward the south.



Lake (MOS-highly contaminated) and control sites at Wowun (WOW) and Straight (STR) Lakes.

The Ontario Ministry of the Environment has monitored the complete spectrum of water quality parameters monthly over the past several years (Hollinger, pers. comm.). The collections have monitored levels of total unfiltered As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn. Levels of Mn, Fe, Cr and Cd exhibited very minor increases, but only copper and zinc have been consistently and significantly elevated over the time of investigation. This study concentrated on monitoring copper and zinc levels. Other data are available from the Ontario Ministry of the Environment (Hollinger, pers. comm.).

Although LMN was selected as an intermediate lake, metal levels in the water declined during the preliminary portions of the study (Table 1) such that MAN and LMN copper levels were not significantly different over the course of this study (Table 2). The copper and zinc concentrations are currently in excess of the Canadian water quality objectives¹, although the values are still substantially below criteria set by the United States Environmental Protection Agency (Table 2). This inconsistency reflects differences in the approaches taken by the two governments to establish their water quality guidelines. The situation provided an ideal location to test an ecosystem health assessment approach for several reasons: contaminated lakes are situated in close proximity to similar uncontaminated, control lakes, metal levels have been elevated for 30 y and alterations should be evident if they are going to occur, an abundance of fish are present and the contaminant levels remain

¹ CCREM (1987) defined a water quality criterion as "data evaluated to derive recommended limits". They define a guideline as "a concentration or statement supporting a designated use" and an objective as "a concentration or statement established to support and protect the designated uses of water". A standard represents "an objective recognized in enforceable environmental laws".

close to the current objectives. If changes are present, they should be near the limit of detection.

Two decisions remained before sampling could begin: the choice of a species for monitoring and the choice of a surveillance level for analysis.

Table 1. Water quality data supplied by the Ontario Ministry of the Environment (Dave Hollinger, pers. comm.). The data was collected monthly during the years 1983-1985 from three sites: a control site (site #24 located on the Black River upstream from Mose Lake), a MAN site (site #25 located near the outflow of Fox Cr.) and a LMN site (site #26 located near the LMN culvert). The values reported here are the yearly means and standard deviations calculated from the monthly data ($\mu\text{g l}^{-1}$).

	Year	MAN	LMN	Control
Alkalinity				
	1985	62.5 \pm 12.2	62.4 \pm 10.4	78.1 \pm 20.8
	1984	67.9 \pm 15.0	63.5 \pm 3.2	79.9 \pm 25.1
	1983	68.2 \pm 14.1	66.5 \pm 10.1	87.2 \pm 17.1
pH				
	1985	6.9 \pm 0.4	7.1 \pm 0.4	7.2 \pm 0.3
	1984	6.9 \pm 0.2	7.2 \pm 0.4	7.2 \pm 0.4
	1983	7.0 \pm 0.2	7.3 \pm 0.4	7.3 \pm 0.4
Hardness				
	1985	112.3 \pm 41.0	108.2 \pm 24.7	85.1 \pm 22.9
	1984	137.3 \pm 45.0	111.6 \pm 23.6	91.0 \pm 23.7
	1983	131.2 \pm 38.2	113.9 \pm 17.3	93.6 \pm 17.7
Copper ¹				
	1985	16 \pm 11	16 \pm 9	2 \pm 3
	1984	34 \pm 30	21 \pm 12	2 \pm 1
	1983	52 \pm 54	22 \pm 13	2 \pm 1
Zinc ¹				
	1985	452 \pm 274	340 \pm 170	4.5 \pm 9
	1984	682 \pm 474	380 \pm 180	2 \pm 1
	1983	1020 \pm 1350	430 \pm 250	4 \pm 2

¹Total unfiltered metals

Table 2. Copper and zinc levels in the water and sediments of the study lakes. Water levels of metals at the contaminated sites exceed Environment Canada's (1984) objectives for both copper (2 ug l^{-1}) and zinc (22 ug l^{-1} ; hardness = 100), but do not surpass the US EPA's criteria for either metal (Cu: 22 ug l^{-1} , Zn: 320 ug l^{-1} , hardness = 100).

Site	Location	1985-1987 Data			German (1971)		
		Cu	Zn	Hardness ¹	Cu	Zn	Hardness
MAN	lake water ²	15.3 ± 4.2 (35)	253 ± 62 (35)	112 ± 41 ⁴	23 (12) ⁵	540 (12)	240
	stream water	13.0 ± 5.3 (6)	400 ± 18 (6)	296 ± 1 (5)	20 (4)	690 ⁶ (4)	380
	sediments ³	102.4 ± 23.6 (18)	1149 ± 328 (18)	-----	791 (6)	4505 ⁷ (6)	---
LMN	lake water	13.3 ± 3.7 (21)	209 ± 20 (21)	108 ± 25 ⁴	14 (5)	290 (5)	137
	sediments	44.4 ± 20.9 (6)	567 ± 273 (6)	-----	ND ⁸	675 (4)	---
LOK	lake water	ND ⁹	26 ± 22 (11)	85 ± 23 ⁴	ND ¹⁰	20 (3)	81
	stream water	4.0 ± 2.7 (3)	3 ± 1 (3)	96 ± 1 (5)	ND	10 (4)	159
	sediments	11.4 ± 2.9 (18)	43 ± 7 (18)	----	ND	ND	---

¹Total hardness (as $\text{mg l}^{-1} \text{CaCO}_3$)

²Total unfiltered metals (as ug l^{-1})

³Total non-residual metals (as mg kg^{-1})

⁴Hollinger (pers. comm.)

⁵sample size in brackets

⁶stream entering Mose Lake 260 and 3410 ug l^{-1}

⁷Fox Bay values as high as 2313 and 7080 mg kg^{-1} respectively

⁸not detectable

⁹less than 2 ug l^{-1}

¹⁰control values from Wowun Lake

2.2 Choice of a Sentinel Species

Although the Manitouwadge chain of lakes supports a diverse ecosystem, several factors have to be considered before the selection of a suitable species for monitoring. Environmental health assessment is retrogressive by nature; the system being monitored acts as the indicator of its own health. Before changes can be detected easily, the time lag between contaminant events and ecological response must be minimized, without altering the ability to detect meaningful changes. The sentinel should not respond unless significant alterations have occurred.

Algal and invertebrate species commonly represent the most toxicant-sensitive biota of aquatic communities. However, the potential replacement of sensitive species, the diversity of community structure, and differences in contaminant tolerance within the community may prohibit expression of toxicant impact in the upper trophic levels. The elimination of one or two toxicant-sensitive species may not alter the overall biomass or the food base for other trophic levels of the ecosystem, necessitating a costly comprehensive study of the entire community structure to isolate direct effects of any contaminants. Diversity within ecosystems can act to buffer minor changes, and the documentation of species loss may not be as important as the identification of the capacity or ability of the system to sustain or maintain the flow of energy.

It appears that monitoring the fish community may offer the most potential for easy detection of meaningful biological impact. Preliminary collections during 1984, together with data from the Ontario Ministry of the Environment (German, 1971) show that a variety of species of fish were present in the lakes, including pike (Esox lucius), walleye (Stizostedion vitreum), white sucker (Catostomus commersoni), yellow perch (Perca flavescens) and lake herring (Coregonus artedii).

Ryder and Edwards (1985) selected the lake trout (Salvelinus namaycush) as a sentinel for following water quality improvements in the Great Lakes. A terminal predator like the lake trout is characterized by a relatively long lifespan with an extended (5-7 y) pre-maturation growth phase. Such characteristics would delay the detection of subtle changes in population characteristics, especially if lower trophic levels of the ecosystem are the first to be affected. Both pike and walleye are terminal predators and are subjected to spring influxes of large numbers of migratory individuals from downstream. Tracing impact in a slowly maturing, top-level predator is difficult enough without having to isolate contaminant effects from those of other stressors (fishing pressure, migration). Clearly, monitoring the upper trophic levels would offer little predictability or opportunity for tracing impact.

The best choice for monitoring would appear to be a species closer to the center of the food web. Abundance and fish size eliminated perch from consideration for this study. Herring are planktivorous and relatively abundant, but could be very difficult to collect from all sites at spawning time. The white sucker is an abundant benthic feeder which congregates in streams at spawning time, facilitating collection. Their exposure to contaminated sediments, intimate interaction with invertebrate species and ease of collection make them an ideal species for monitoring. Most of Ryder and Edwards' (1985) selection criteria for a sentinel are satisfied by the white sucker: they are a widely distributed, indigenous fish which constitute a stable, structural component of the food web. The habitat requirements and niche characteristics of suckers are known and they are suitable for laboratory experimentation. Furthermore, suckers have been shown to respond to elevations of contaminants (McFarlane and Franzin, 1978; Duncan and Klaverkamp, 1983;

Schmitt et al., 1984) and, as benthic foragers, they have previously received some attention as a possible environmental sentinel. As a primary screening tool for toxicant-sensitive or high-risk areas, monitoring sucker populations may be the least expensive and most attractive method for first approximation of impact.

2.3 Surveillance Level

Once the sentinel had been chosen, decisions had to be made regarding the level of monitoring to use. Ecosystem health can be assessed by monitoring a species in two ways: a "top-down" (holistic) approach or a "bottom-up" (reductionist) approach. Both approaches have advantages and disadvantages (Hodson, 1987b). The holistic approach monitors populations or communities of organisms for evidence of adverse effects. A limitation of this approach is the time lag which occurs between contaminant events and the ability to identify changes. This time lag could be shortened considerably through the development of appropriate biochemical testing (NRCC, 1985; Hodson, 1987a,b), since all chemical effects on ecosystems begin with an interaction between a contaminant and a biochemical reaction within an individual (Hodson, 1986). There has been much emphasis on the development of biochemical indicators of toxicant impact. Many biochemical tests are thought to hold some promise (Klontz, 1984) and indicators have been developed for a limited number of cases where cause and effect have been demonstrated (NRCC, 1985; Dixon et al., 1987; Luxon et al., 1987).

Although reductionist monitoring offers an increased early-warning capability, dose-response sensitivity and increased potential for causal demonstration (Hodson, 1986), the alteration of biological substrates is not necessarily indicative of lasting

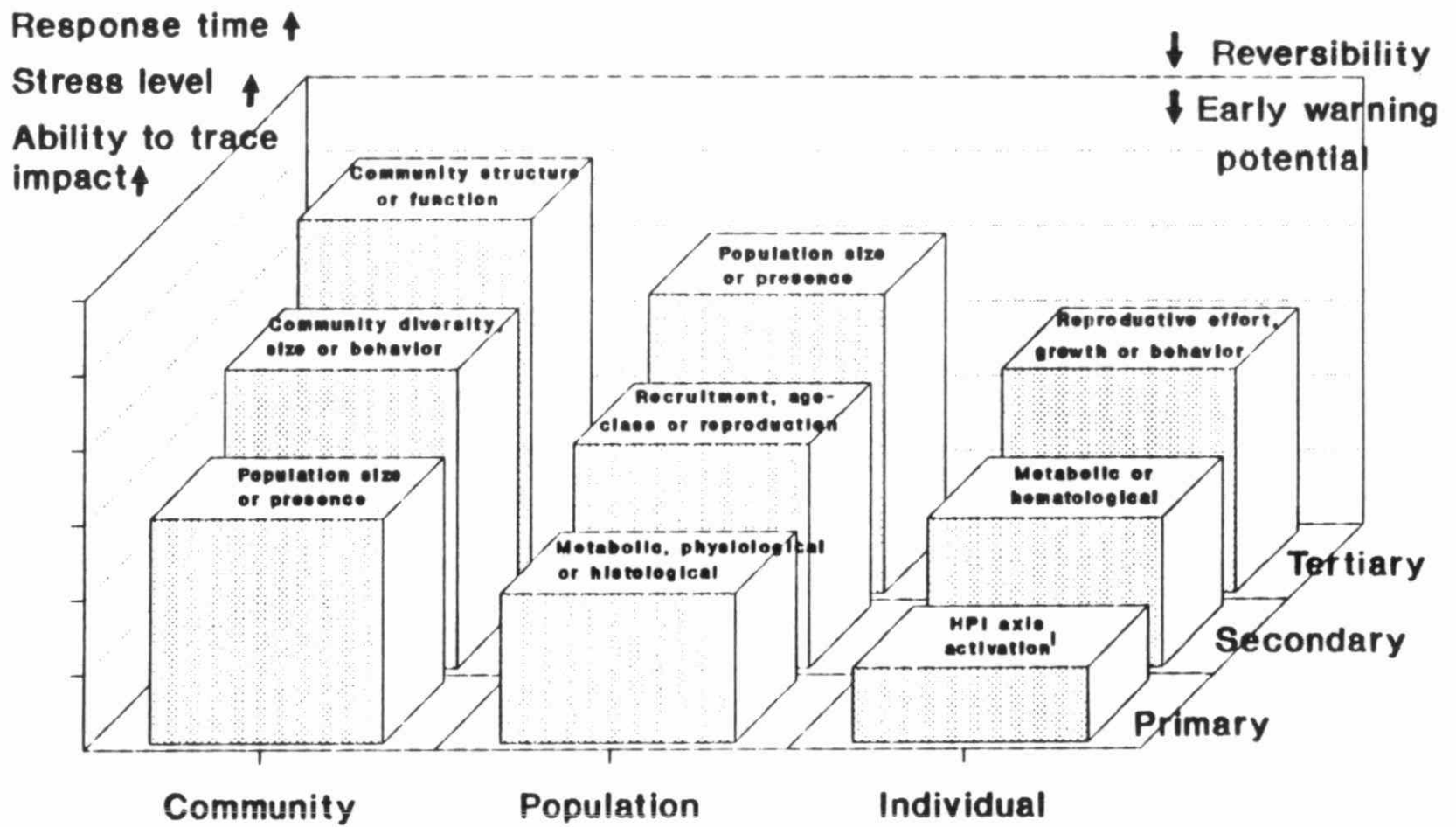
changes at the whole organism level. The dependence of a monitoring program on the acute stress response of individual organisms would result in many positive responses, although a large number of cases would fail to show meaningful, lasting alterations at the level of the whole organism. Monitoring changes at the community level would fail to detect changes in many affected ecosystems due to the associated time lag before demonstration of effect. A compromise would be the surveillance of ecosystems at a level offering evidence of changes at both the individual and community level.

The stress response characteristically consists of primary, secondary and tertiary responses at the level of the individual, population and community (reviewed in Pickering, 1981; Cairns et al., 1984). At the individual level, primary and secondary changes are easily reversible, generalized responses which can lack long-lasting effects at the whole organism level. Tertiary, individual effects are not as reversible (Figure 2), consist of changes associated with reproduction, disease resistance and survival and seem to be the most meaningful in terms of population effects. Organismic changes work through individuals to result in changes in the overall characteristics of populations (Kerr and Dickie, 1984; Wedemeyer et al., 1984). Tertiary, individual changes would result in secondary changes at the population level and primary changes at the ecosystem level (Figure 2). Again, primary responses are the most reversible at the community level.

Figure 2:

Stress-response matrix (modified from Rapport, 1984; NRCC, 1985). Although the matrix suggests causative links between steps, it must be emphasized that such links have not been positively identified in all cases (Leatherland and Sonstegard, 1984; Wedemeyer et al., 1984). The smallest box represents a response to a low level of stress, which has the fastest response time, the most reversibility, best early warning potential and worst diagnostic potential. The largest box represents a response to the largest amount of stress, which has the least reversibility, the longest response time, the best diagnostic potential and the lowest early warning potential.

¹HPI-Hypothalamo-pituitary-interrenal axis (Donaldson et al., 1984).



2.4 Scope of the Study

In laboratory testing, the most contaminant-sensitive aspects of a fish's life history consistently appear to be growth, reproduction and larval survival (McKim 1977; Bresch 1982; Donaldson and Scherer 1984; Woltering 1984; Norberg and Mount 1985; Neuhold, 1987). This study will concentrate on general population indicators of ecosystem health which integrate lower level effects and have some predictive value for higher level effects. This study was initiated to determine whether the toxicological impacts of mixed copper and zinc contamination in several lakes of the Manitouwadge district of Northern Ontario could be detected using an ecosystem health assessment model based on the growth and reproductive capacity of indigenous populations of white sucker.

Chapter III

GROWTH AND REPRODUCTION

3.1 Introduction

Growth and reproduction are among the most toxicant-sensitive aspects of a fish's life history. Metals have been associated with changes in both growth (Lett et al., 1976; Collvin, 1985) and reproduction (McKim and Benoit, 1974; Benoit, 1975; Benoit and Holcombe, 1978), and both responses incorporate a fish's experience over a considerable period of time into factors which are measureable with a minimum of equipment and expertise. Population data based on maturity, fecundity and year-class strengths offer insight into population fluctuations over the past several years.

McFarlane and Franzin (1978) examined sucker populations in the vicinity of a heavy metal smelter at Flin Flon, Manitoba, and found that suckers exposed to copper, zinc and cadmium exhibited increased fecundity, increased growth rates and an increased incidence of spawning failure. Levels of both copper and zinc in the water of their study lakes was almost identical to the MAN site, so this study set out to examine the growth performance and reproductive development of suckers in the Manitouwadge chain of lakes. The purpose of the collections was to attempt to examine physiological phenomena associated with the increased performance of suckers under adverse environmental conditions. Biochemical and physiological testing was used to attempt to identify the target site associated with alterations.

3.2 Methods

3.2.1 Collections

The growth and reproductive capacity of white sucker populations from control and metal-contaminated sites were assessed in terms of age, length, weight, condition factor, fecundity and physiological indicators of lipid status. Manitouwadge Lake (MAN) and Little Manitouwadge Lake (LMN) were used as the metal-contaminated sites and Loken Lake (LOK) as the control site. Fish were collected from MAN, LMN and LOK with 8.8 cm monofilament gill net during the prespawning (PRES), postspawning (POST) and fall recrudescence (RECR) periods from 1985 to 1987. To examine growth effects at another contaminated site, additional samples were collected from Mose Lake (MOS) during the postspawning periods of 1986 and 1987. A limited number of samples were collected from an alternate control site (Wowun Lake) during the postspawning period of 1987. Fish were collected from the gillnets every 45-60 min, transported to shore in an adequate volume of water, and sampled as quickly as possible. During spawning (SPAW), fish were collected by dipnet and sampled as quickly as possible.

3.2.2 Necropsy

At no time were moribund fish sampled; effects of capture stress on the physiology of the fish were assumed to be equal at all sites. Fish were anesthetized in a solution of tricaine methanesulphonate (MS222), and examined for external lesions. The weight (± 5 g) and standard length (± 0.5 cm) of each fish were recorded. A condition factor (k) was calculated as $100(\text{weight}/\text{length}^3)$. The left pectoral ray of each fish was removed at the base, dried, sectioned with a 7/0 jeweller's saw and

the annuli counted to determine age. Scales were removed from the left side of each fish above the lateral line for comparison of age determination.

Blood was collected via cardiac puncture using either a 4.0 ml syringe or a 5.0 ml vacuutainer, both with a 21 ga. needle. Blood was clotted on ice for 6 to 8 hr and spun for 7 min on a Janetzki T5 centrifuge. The sera was collected with a Pasteur pipette, placed in plastic beem capsules, frozen in liquid nitrogen and stored at either -70°C or -20°C until analysis.

The peritoneal cavity was opened and organs were examined for abnormalities. The ovaries were removed, weighed (± 0.1 g), and preserved in 10% buffered formalin. A gonadosomatic index was calculated as $100(\text{gonad weight/body weight})$. After storage, the ovaries were washed, blotted dry and weighed (± 0.001 g). The number of eggs in duplicate 1 g samples were determined and the results were used to estimate the total number of eggs per fish. For each fish, the diameter of 10 eggs and the weight of samples of 10 eggs were also determined.

The viscera of the fish were removed and the liver was separated from the intestine. The liver was weighed (± 0.1 g) and samples of both liver and muscle were frozen in liquid nitrogen and stored at -20°C . The liversomatic index (LSI) was calculated as $100(\text{liver weight/body weight})$. The number of parasitic acanthocephalan worms protruding through the wall of the intestine were recorded on a subjective scale with 0 being none, 1 less than 50 and 2 more than 50. Fish were also rated with respect to their visceral lipid stores during autumn (RECR) sampling periods (Table 3).

Table 3. Subjective rating scale for visceral lipid stores of white suckers collected during the fall recrudescence period.

Rating	Description
1	Very thin band (<5 mm) along the intestine with very little along the gonads.
2	Thin (~5 mm) strip along the dorsal region of both gonads and a smaller strip along the intestine.
3	Lipids forming a thin layer near the body wall and extending over the posterior intestine.
4	Posterior intestine covered in lipid material which extends across the dorsal body wall.
5	Intestine totally encased in lipids with a layer around the peritoneum.

3.2.3 Biochemical analyses

Liver glycogen stores were determined after hydrolysis by a modified (Passonneau and Lauderdale, 1974) serum glucose technique (Boehringer-Mannheim Co., Quebec). Liver and muscle total lipid estimates were performed on duplicate samples (0.1 and 1.0 g wet weight respectively) by a modified (Herbes and Allan, 1983) chloroform:methanol extraction (Bligh and Dyer, 1959). Total serum lipids were measured using a spectrophotometric analysis of a phosphoric acid:vanillin reaction (Boehringer Mannheim Co., Quebec) at a wavelength of 535 nm. Total serum protein was analyzed using the Biuret method (Sigma Chemical Co., St. Louis, MO) at a wavelength of 545 nm. Serum triglycerides, total cholesterol and high-density lipoprotein cholesterol (HDL) were measured with Sigma kits at wavelengths of 410 nm, 500 and 500 nm respectively.

Serum calcium was analyzed by atomic absorption spectrophotometry using lanthanum to eliminate phosphorus interference (Beamish et al., 1975). Serum levels of alkali-labile, protein-bound phosphorus were measured after precipitation of the serum proteins with 5 ml of cold 10% (w/v) trichloroacetic acid. The precipitate was washed progressively with hot (80°C) ethanol, and room temperature mixtures of chloroform:ether:ethanol (1:2:2), acetone and ether. The washed precipitate was incubated in 1 ml of 2N NaOH for 15 min at 100°C, and neutralized with 1 ml of 2N HCl. The solution was analyzed for phosphorus content with a Sigma kit at 660 nm.

3.2.4 Statistics

Data were examined for normality and homoscedasticity, and compared using analysis of variance procedures available through the University of Waterloo SAS and SYSTAT packages. When main effects or interactions were significant, a conservative least square means or modified Tukey's test were used to compare treatment means (Zar, 1984). Length, weight and age regressions, as well as tests involving condition factor, serum calcium and serum phosphorus levels, were conducted on log-transformed values. Subjective ratings of visceral lipids, parasite load, spawning failure and sex ratio were compared using non-parametric, loglinear procedures for three-dimensional contingency tables. In all cases, significance levels were set at $p=0.05$.

3.3 Results

Female fish were significantly longer ($p<.0001$) and heavier ($p=.0008$) than male fish and females from control sites were significantly longer and heavier than females from contaminated sites ($p<.0001$) (Table 4). The only significant difference among male fish was evident during the POST period; LOK males were significantly longer than males from contaminated sites ($p<.05$). There was a marginal interaction between sex and season for condition factor ($p=.059$). Condition factors of male fish varied with site ($p=.0007$) and season ($p=.015$), and the condition factors of both LOK and LMN were significantly larger than MAN. Male condition factors during RECR were significantly lower than PRES ($p<.05$). There were no effects of either site ($p=.079$) or season ($p=.564$) on the condition factors of female fish (Table 5).

Table 4. Length and weight of fish collected from the study lakes.
Values are shown as mean \pm s.e. (sample size).

Site	Length (cm)	Weight (g)
<i>A. Males</i>		
MAN	32.4 \pm 0.3 (55)	693.1 \pm 18.0 (51)
LMN	32.6 \pm 0.2 (66)	739.4 \pm 18.6 (66)
LOK	32.8 \pm 0.4 (43)	763.1 \pm 26.6 (43)
<i>B. Females</i>		
MAN	33.4 \pm 0.3 (78)	775.6 \pm 21.9 (72)
LMN	33.4 \pm 0.3 (64)	791.3 \pm 20.2 (63)
LOK	35.2 \pm 0.4 (88)	963.1 \pm 35.8 (88)

Table 5. Age (A), condition factor (K, B), liver somatic index (LSI, C) and gonadosomatic index (GSI, D) of white suckers collected from control (LOK) and metal-contaminated (MAN, LMN) lakes during different phases (PRES, POST, RECR) of the reproductive cycle. Values are given as means (S.E., n). For age, means sharing a superscript are not significantly different. The statistical significance of the data for K, LSI and GSI are given in the results section.

Lake	Male			Female			Site mean
	PRES	POST	RECR	PRES	POST	RECR	
<u>A. Age(y)</u>							
LOK	6.0 (0.4, 19) ^b	7.7 (0.7, 9) ^a	4.5 (0.4, 8) ^c	6.2 (0.4, 25) ^b	6.0 (0.5, 20) ^b	7.0 (0.3, 29) ^b	6.34 (0.20, 110) ^a
MAN	7.6 (0.3, 23) ^a	6.1 (0.5, 13) ^b	6.3 (0.8, 8) ^b	7.4 (0.4, 19) ^a	6.0 (0.4, 22) ^b	5.8 (0.4, 21) ^b	6.58 (0.18, 106) ^a
LMN	7.5 (0.3, 20) ^a	7.7 (0.3, 28) ^a	7.1 (0.5, 17) ^b	7.2 (0.2, 26) ^b	6.9 (0.4, 22) ^b	5.5 (0.4, 12) ^c	7.13 (0.15, 125) ^b
<u>B. K</u>							
LOK	2.11 (0.04, 19)	2.15 (0.07, 10)	2.18 (0.04, 14)	2.18 (0.03, 26)	2.21 (0.05, 29)	2.09 (0.04, 33)	2.15 (0.02, 131)
MAN	1.97 (0.03, 23)	2.04 (0.06, 14)	2.05 (0.05, 14)	2.04 (0.04, 20)	2.15 (0.06, 22)	2.07 (0.04, 29)	2.05 (0.02, 122)
LMN	2.06 (0.05, 20)	2.11 (0.05, 29)	2.22 (0.05, 17)	2.15 (0.04, 25)	2.03 (0.04, 25)	2.12 (0.06, 13)	2.11 (0.02, 129)
Season mean	2.04 (0.03, 62)	2.10 (0.04, 53)	2.15 (0.03, 45)	2.13 (0.02, 71)	2.13 (0.03, 76)	2.09 (0.03, 75)	
<u>C. LSI</u>							
LOK	1.29 (0.08, 9)	1.52 (-, 1)	0.95 (0.06, 11)	1.49 (0.07, 9)	1.66 (0.07, 9)	1.14 (0.06, 15)	1.28 (0.04, 54)
MAN	1.50 (0.08, 13)	1.56 (0.13, 6)	1.07 (0.09, 10)	1.68 (0.09, 9)	1.71 (0.12, 7)	1.31 (0.07, 19)	1.43 (0.05, 64)
LMN	1.50 (0.09, 14)	1.48 (0.07, 8)	1.11 (0.10, 9)	1.69 (0.05, 13)	1.82 (0.15, 9)	1.48 (0.15, 8)	1.52 (0.05, 61)
Season mean	1.45 (0.05, 36)	1.51 (0.07, 15)	1.04 (0.05, 30)	1.63 (0.04, 31)	1.73 (0.07, 25)	1.28 (0.05, 42)	
<u>D. GSI</u>							
LOK	4.0 (0.2, 19)	1.4 (0.2, 10)	6.0 (0.3, 14)	11.7 (0.6, 18)	1.9 (0.5, 25)	4.3 (0.3, 24)	-
MAN	4.4 (0.2, 22)	2.4 (0.2, 14)	6.3 (0.3, 14)	11.9 (0.4, 20)	5.1 (1.1, 22) ¹	4.6 (0.3, 29)	-
LMN	4.3 (0.1, 20)	1.7 (0.1, 26)	6.2 (0.5, 17)	10.8 (0.5, 25)	1.6 (0.1, 21)	4.3 (0.3, 13)	-

1. Includes fish which failed to spawn. The mean with those fish removed was 1.9.

Collections during different seasons showed variations in the age of fish ($p < 0.0001$). Overall, the age of MAN fish was not significantly different from LOK fish, although LMN fish were slightly older ($p = 0.009$; Table 5), and the age distribution of MAN fish was shifted slightly towards older fish (Figure 3). At LMN, male fish were significantly older than females ($p = 0.0026$), although there was no effect of sex on age at either MAN ($p = 0.54$) or LOK ($p = 0.42$). During spawning, the migration of fish to the nearshore areas altered the age distribution of collections and the ratio of males to females at all sites (Table 6). The effects on age distribution varied with site, and at MAN, PRES suckers were significantly older than POST or RECR fish ($p = 0.0003$), while at LMN, both PRES and POST suckers were older than RECR ($p = 0.013$) and at LOK, POST fish were older than both PRES and RECR ($p = 0.0013$). Despite seasonal changes, the sex ratio of MAN and LOK fish were not significantly different ($p = 0.123$), although both were significantly different from LMN ($p < 0.001$). There was a significant sex and reproductive stage interaction on the sex ratios of the fish ($p = 0.015$).

At ages 4 and 5, there were no significant differences in either length or weight between the control and contaminated sites, but LOK females were significantly longer and heavier than females from contaminated sites at ages of 7, 8 and 9 (Figure 4). Fish older than 10 years of age were pooled because of their low frequency, and LOK fish in this age group were significantly longer ($p < 0.0001$) and heavier ($p = 0.0003$) than fish from contaminated sites (Table 7). LOK fish exhibited significant linear relationships between age and both length and weight for male and female fish (Table 8). There were no significant effects of age on weight of female fish at any contaminated site, and only MAN females exhibited a significant rela-

Table 6. Sex ratios (M:F) of white suckers collected from control (LOK) and metal-contaminated (MAN, LMN) lakes during different phases of the reproductive cycle. Values sharing a superscript are not significantly different.

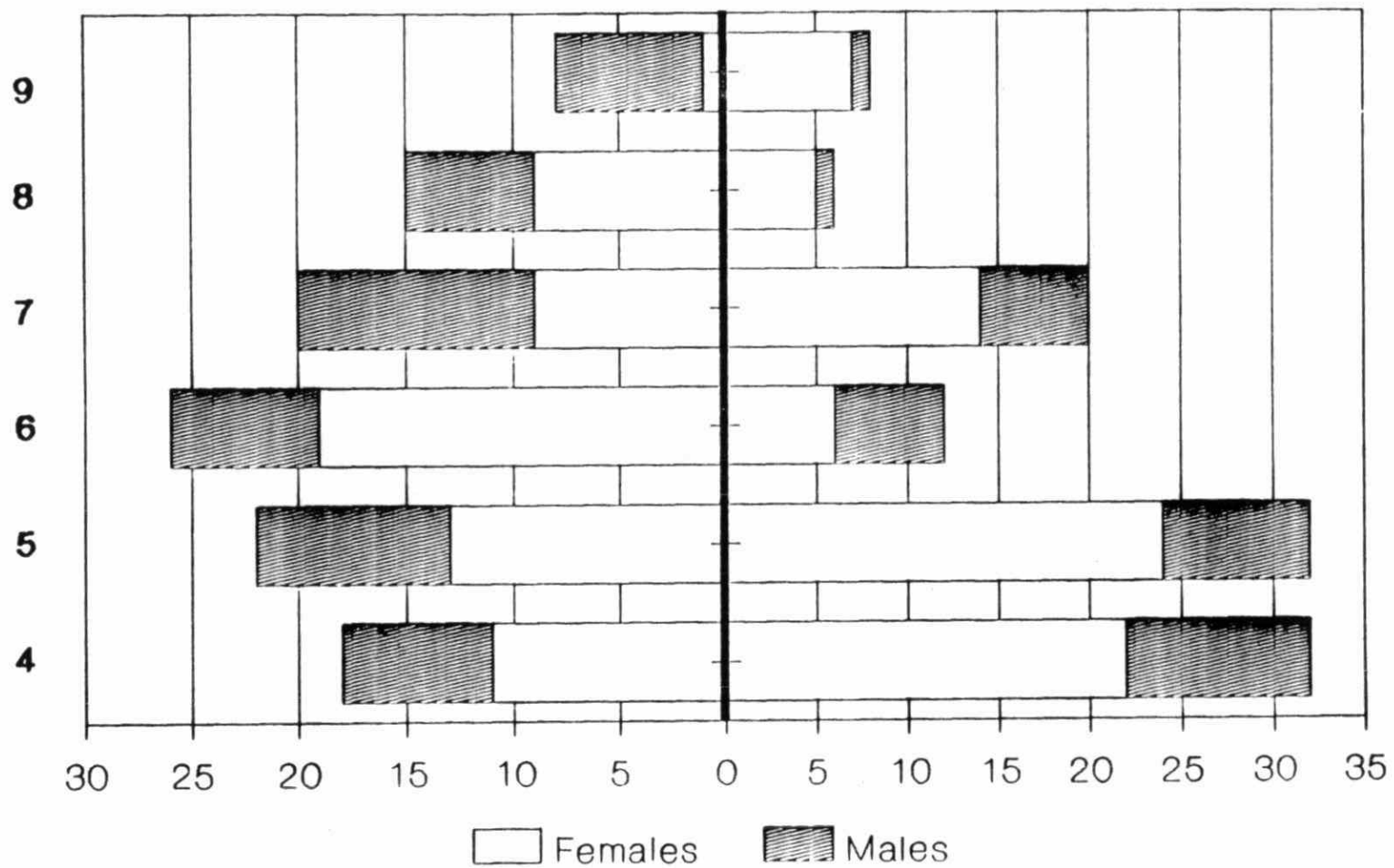
Site	PRES	POST	RECR	Total
LOK	30:34	15:41	14:33	59:108 ^a
MAN	24:22	21:29	14:30	59:81 ^a
LMN	35:26	42:30	42:13	119:69 ^b

Figure 3: Age distributions of fish from MAN and LOK. Data represents pooled values for both sexes, females are represented by shaded portions of boxes and males by the open portions.

AGE

MAN

LOK



tionship between length and age (Table 8). Despite this relationship, the slope was significantly lower than LOK fish ($p < .05$), and the correlation coefficient (r^2) was less than 0.10 (compared to 0.61 for LOK). Effects on males were not as obvious (Figure 5) and regressions of age on length and weight of MAN and LMN males were not significantly different from LOK fish.

At LOK, 40.6% (13/32) of the 4 and 5 year old females captured during PRES were immature. Using Lysack's formula (Tripple and Harvey, 1987b), the age of maturation (Z_{age}) of LOK females was 4.8 y compared to 4.1 y for MAN. The estimates of reproductive life span ($RLS = x_{age} - Z_{age}$) of females were 2.33 y for LOK and 2.47 y for MAN. MAN suckers exhibited an increased incidence of spawning failure among females between the ages of 4 and 7 (7/22) compared to LOK fish (3/40) ($p = .033$). All LOK females which failed to spawn were aged 5 y, while at MAN the average was 5.9 y. One additional MAN female of age 10 y also failed to spawn. There was a highly significant effect of season on the gonadosomatic index (GSI) ($p < .0001$). There was no significant effect of site on the GSI of either male ($p = .299$) or female fish ($p = .355$), if female fish which failed to spawn were removed from the comparison (Table 5). There was also no difference detected in the stages of egg development between sites (unpubl. data).

The weight of LOK eggs (18.7 ± 0.3 mg) was significantly more than eggs from the MAN site (17.2 ± 0.3 mg) ($p < .05$). LOK fish also had significantly more eggs ($\bar{x}, s.e., n$: 31200, 2619, 19) than fish from either MAN (22635, 746, 26) or LMN (21863, 958, 22) ($p < .0001$). The effect of site on fecundity was significant in all relationships of fecundity using length, weight and/or age as covariates. Fecundity was significantly correlated with both length and weight at all sites, although

Figure 4: Weight and age of female suckers. Values are shown for LOK fish (open squares), MAN (closed circles) and LMN (open circles), with the sample size and standard errors. Fish were aged by pectoral ray sections.

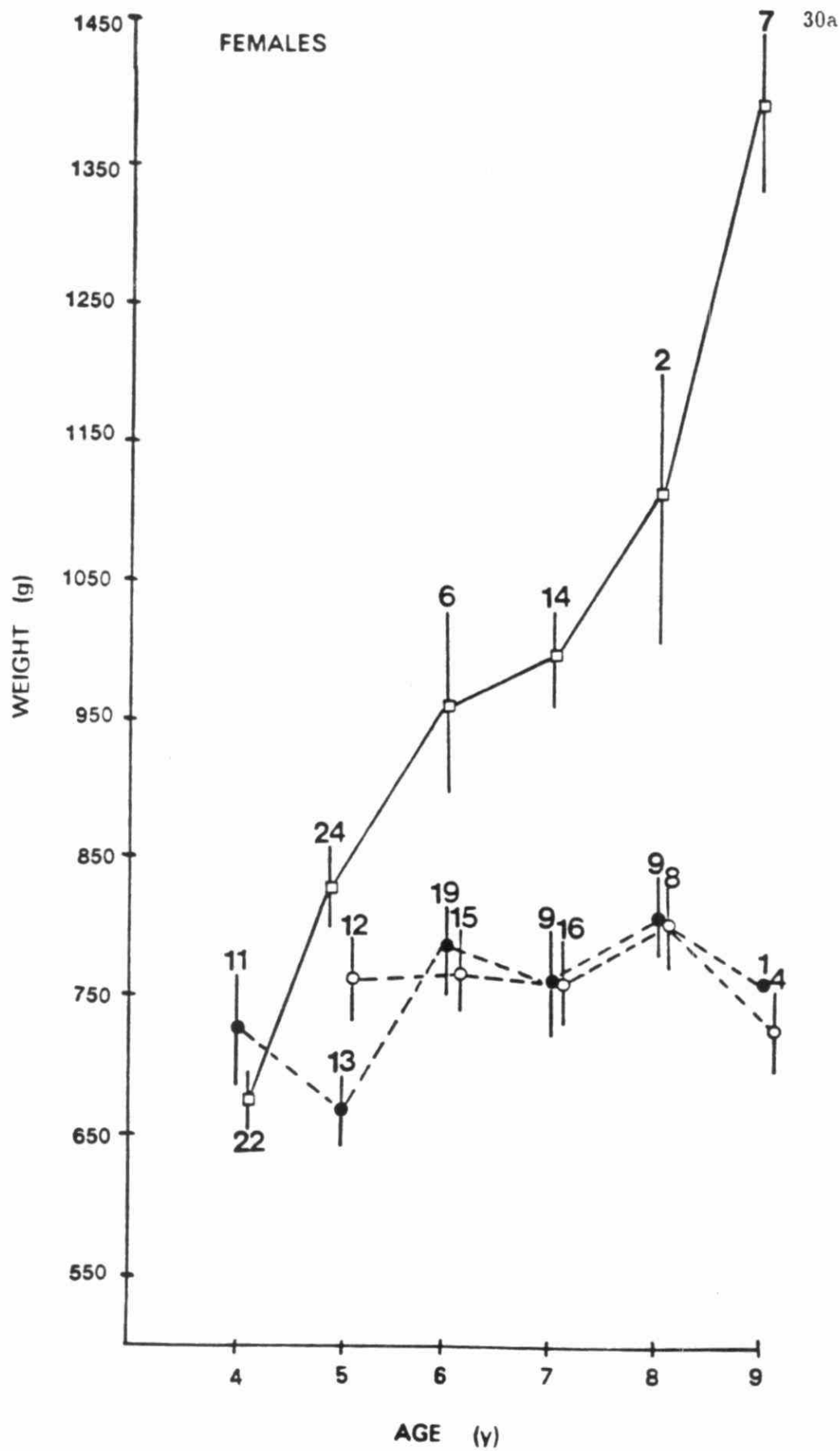


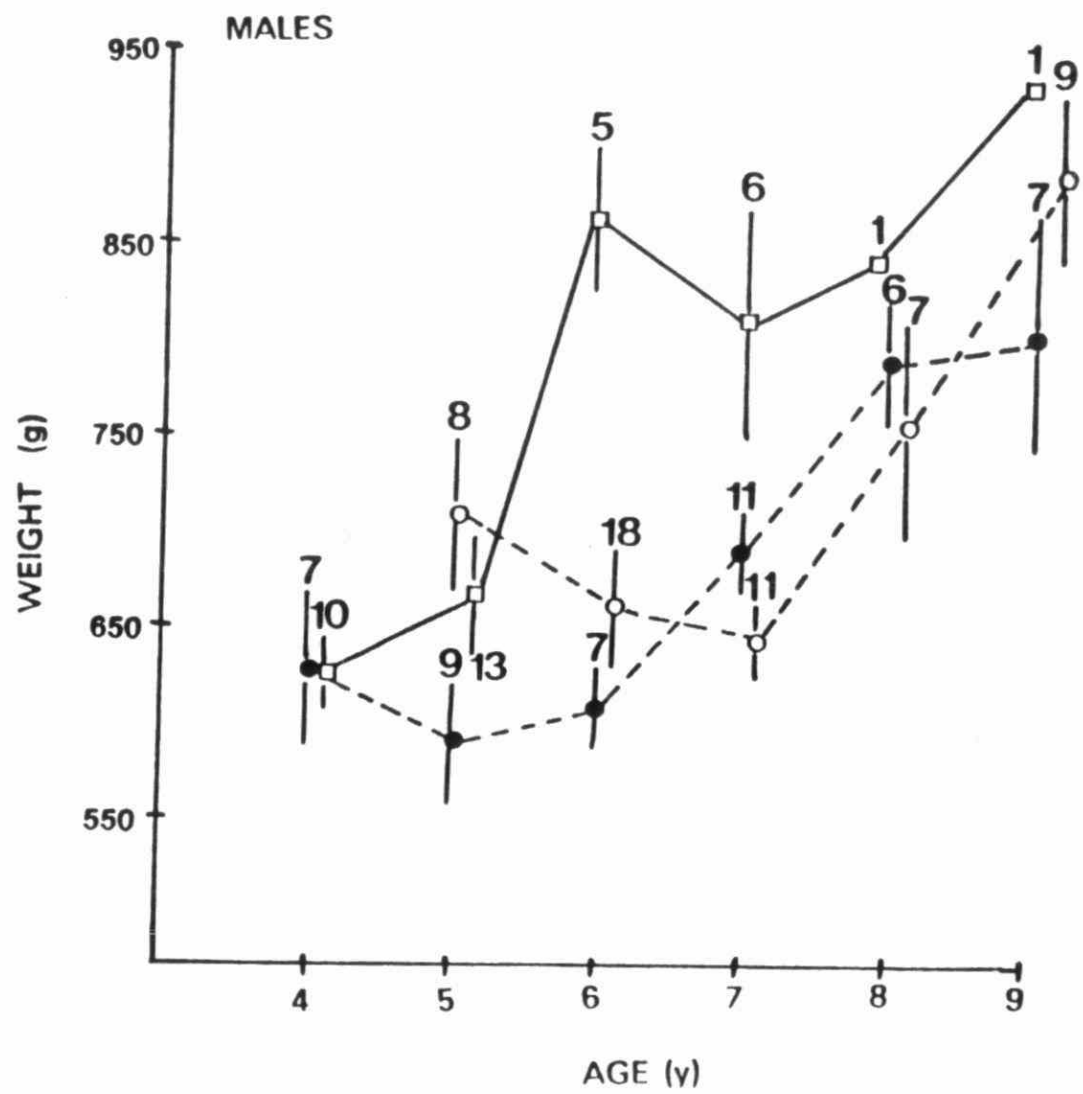
Table 7. Length and weight of all fish 10 yr of age or older.

Site	Male		Female	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)
MAN	35.0 \pm 1.0 (3)	891.7 \pm 76.2 (12)	37.6 \pm 1.3 (7)	1067 \pm 88 (7)
LMN	33.6 \pm 0.6 (12)	823.3 \pm 47.4 (12)	40.8 \pm 0.7 (3)	1272 \pm 104 (3)
LOK	37.0 \pm 1.0 (5)	1021 \pm 69 (5)	41.5 \pm 0.7 (12)	1469 \pm 112 (12)

Table 8. Characteristics of regressions for loglength (A) and logweight (B) against age of suckers from control (LOK) and metal-contaminated lakes (MAN, LMN, MOS). For slopes and intercepts, values within a column sharing a superscript are not significantly different. An asterisk signifies that the regression was not significant.

Sex	Site	Slope	p(B=0)	Intercept	p(int=0)	r ²	n
<u>A. Loglength</u>							
M	MAN	0.0101 ^a	<0.0001	1.444 ^b	<0.0001	0.41	47
M	LMN	0.0095 ^a	0.0001	1.444 ^b	<0.0001	0.25	52
M	MOS	0.0063	0.52	1.470	*	0.06	9
M	LOK	0.0111 ^a	<0.0001	1.446 ^b	<0.0001	0.39	37
F	MAN	0.0056 ^b	0.0123	1.485 ^b	<0.0001	0.98	63
F	LMN	-0.0006	0.79	1.520	*	0.001	53
F	MOS	0.0044	0.44	1.510	*	0.041	17
F	LOK	0.0202 ^c	<0.0001	1.420 ^b	<0.0001	0.610	76
<u>B. Logweight</u>							
M	MAN	0.0275 ^a	<0.0001	2.65 ^b	<0.0001	0.37	47
M	LMN	0.0218 ^a	0.0001	2.70 ^b	<0.0001	0.14	47
M	MOS	0.0161	0.399	2.74	*	0.10	9
M	LOK	0.0367 ^a	<0.0001	2.65 ^b	<0.0001	0.39	37
F	MAN	0.0115 ^b	0.067	2.81 ^a	<0.0001	0.054	63
F	LMN	-0.0033	0.66	2.91	*	0.004	53
F	MOS	0.017	0.285	2.79	*	0.076	17
F	LOK	0.0587 ^c	<0.0001	2.60 ^b	<0.0001	0.64	76

Figure 5: Weight and age of male suckers. Values are shown for LOK (open squares) and for MAN (closed circles) and LMN (open circles), with the sample size and standard error bars.



the correlations were significantly stronger at LOK (Table 9). Fecundity was also highly correlated with the age of LOK fish ($p < .001$), but exhibited no relationship with age at either contaminated site (Figure 6).

There were significant effects of sex, site and season on the liversomatic index (LSI). Female fish had a larger LSI than males ($p < .0001$), and the LSI of LOK fish was significantly smaller than either LMN or MAN fish ($p = .0056$) (Table 4). RECR LSIs were significantly smaller than either PRES or POST values ($p < .0001$). The LOK site exhibited a significantly higher prevalence and intensity of infection with intestinal acanthocephalans than found at two contaminated sites ($p < .0001$, Table 10). There was no effect of sex on parasite burden ($p = .56$).

At the contaminated site, female fish exhibited obvious effects on growth and fecundity, suggesting that differences in energy stores or lipid metabolism may be evident. Estimates of total muscle lipids during the RECR period found there to be no effect of sex ($p = .51$) and a marginal interaction between sex and site ($p = .057$). LOK females exhibited significantly higher muscle lipid levels than did MAN females ($p = .004$), although there was no difference between males ($p = .90$) (Table 11). The differences in female muscle lipid levels were consistent during different seasons ($p = .92$), and MAN levels were significantly lower than LOK during both the POST and RECR periods ($p = .039$).

Visual observations of visceral lipid stores during the fall collection period suggested a decrease in lipid stores at contaminated sites entering the overwintering period. However, statistical analysis could not detect an effect of site ($p = .56$), even though only 1/17 females at the contaminated site reached a value of 3 on the subjective scale (Table 3). Analysis was complicated by an interaction between the sex

Table 9. Coefficients for relationships of fecundity (total number of eggs) with age (y, A), length (cm, B) and weight (g, C) of white suckers from control (LOK) and metal-contaminated (MAN, LMN) lakes. An asterisk denotes that the regression was not significant.

Lake	Slope	p(B=0)	Intercept	p(int=0)	r ²	n
<u>A. Age</u>						
LOK	4602.4	0.0001	-47.7	<0.001	0.660	19
LMN	1183.8	0.226	13310	*	0.072	22
MAN	741.8	0.093	17613	*	0.118	25
<u>B. Length</u>						
LOK	2259.0	0.0001	-49708	<0.001	0.880	19
LMN	1526.9	0.0003	-29565	<0.001	0.489	22
MAN	1092.4	0.0005	-13602	<0.001	0.399	26
<u>C. Weight</u>						
LOK	25.92	0.0001	3454	<0.001	0.951	19
LMN	20.88	0.0001	4679	<0.001	0.641	22
MAN	22.68	0.0001	5219	<0.001	0.510	26

Figure 6: Fecundity (total # eggs) of white suckers of different ages. Fish were collected from LOK (closed circles), MAN (open circles) and LMN (triangles). Only the relationship involving LOK fish was significant ($r^2=.66$).

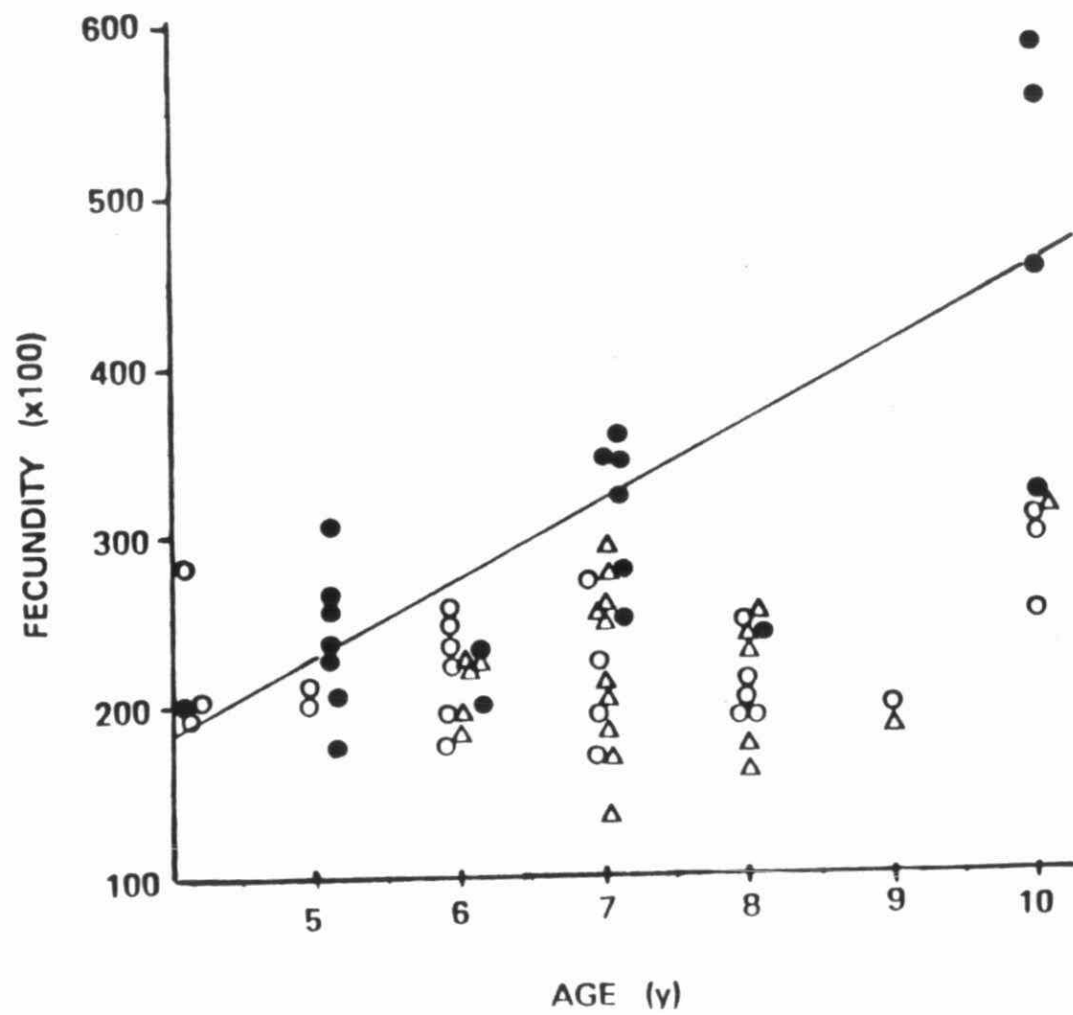


Table 10. Subjective estimates of visceral lipid stores (A) and the incidence of parasitic acanthocephalans (B) for white suckers from control (LOK) and metal-contaminated (MAN, MOS) sites. Values are given as mean \pm s.e. (sample size). Values within columns sharing a superscript are not significantly different. All estimates were made during the RECR period of 1986.

Site	Male	Female	Mean	Incidence (%)
<u>A. Lipids</u>				
MAN	1.7 \pm 0.3 (6)	2.3 \pm 0.2 (8)	2.0 \pm 0.2 (14)	---
MOS	1.6 \pm 0.2 (10)	2.3 \pm 0.3 (10)	1.9 \pm 0.8 (20)	---
LOK	1.6 \pm 0.2 (6)	3.1 \pm 0.5 (8)	2.7 \pm 0.4 (14)	---
<u>B. Parasites</u>				
MAN	0.85 \pm 0.40 (6)	0.25 \pm 0.16 (8)	0.5 \pm 0.2 (14) ^a	35 ^a
MOS	0.60 \pm 0.33 (10)	1.10 \pm 0.48 (10)	0.9 \pm 0.3 (20) ^a	35 ^a
LOK	3.67 \pm 0.33 (6)	3.13 \pm 0.51 (8)	3.4 \pm 0.3 (14) ^b	93 ^b

of fish and the visceral fat estimate ($p=.02$). Data from attempts to accurately weigh the deposits under field conditions were too variable to be of use.

Examinations of other quantitative estimates of lipid status failed to detect many differences at the contaminated sites. There were significant interactions of sex and season for measurements of liver lipids ($p=.012$), liver glycogen ($p<.0001$), serum lipids ($p=.0001$), serum triglycerides ($p=.0004$) and total cholesterol ($p=.0012$). Due to the interactions with sex of fish, male and female values were analyzed separately.

In male fish there was no effect of site on liver lipids ($p=.06$), liver glycogen ($p=.297$), serum lipids ($p=.31$), serum triglycerides ($p=.47$), or total cholesterol ($p=.35$), but seasonal changes were found in all cases except cholesterol ($p=.445$). PRES levels of liver lipids were significantly higher than RECR ($p=.010$) (Figure 7), PRES liver glycogen were elevated over both POST and RECR levels ($p=.0001$) (Table 11), PRES triglycerides were higher than SPAW or RECR ($p=.008$) and SPAW levels of serum lipids were higher than either POST or RECR ($p=.0006$) (Table 12).

In female fish, total cholesterol levels were significantly higher at MAN than at LOK ($p=.007$). Total serum lipids exhibited a marginal interaction between site and season ($p=.06$) due to a significant POST difference between sites ($p=.0097$) (Figure 7). No effects of site were detected on liver lipids ($p=.188$), liver glycogen ($p=.63$) or serum triglycerides ($p=.26$). POST levels of liver lipids and glycogen were elevated over PRES and RECR ($p=.002$, $.004$). Similarly, serum triglyceride levels during POST were elevated over SPAW and PRES periods. Total serum cholesterol levels during RECR were higher than all other seasons ($p<.0001$) (Figure 8).

Table 11. Levels of liver glycogen, liver lipids and muscle lipids of white suckers collected from control (LOK) and metal-contaminated (MAN) lakes during different phases (PRES, POST, RECR) of the reproductive cycle. Values are given as means (S.E., n). Values sharing a superscript within a sex are not significantly different.

Sex	Phase	Liver glycogen (mg g ⁻¹)		Liver lipids (mg g ⁻¹)		Muscle lipids (mg g ⁻¹)	
		MAN	LOK	MAN	LOK	MAN	LOK
M	PRES	72.1 (3.2,8) ^a	45.5 (10.4,7) ^a	63.0 (8.0,9) ^a	47.1 (10.0,3) ^a	-	-
M	POST	34.7 (8.8,8) ^b	38.4 (7.8, 5) ^b	51.0 (6.9,7) ^{a,b}	46.7 (5.8,7) ^{a,b}	-	-
M	RECR	23.8 (4.8,7) ^b	28.1 (4.9, 7) ^b	41.7 (3.7,12) ^b	33.1 (2.1,13) ^b	7.4 (0.4,9) ^a	7.1 (0.3,5) ^a
F	PRES	22.0 (5.6,9) ^a	23.9 (6.0,10) ^a	27.5 (3.7,9) ^a	33.2 (4.7,8) ^a	-	-
F	POST	41.3 (5.5,10) ^b	37.8 (7.0,12) ^b	41.4 (8.9,9) ^b	49.3 (5.6,12) ^b	6.2 (0.5,8) ^a	8.9 (1.0,8) ^b
F	RECR	18.1 (4.9,11) ^a	26.4 (3.3,10) ^a	33.0 (2.2,20) ^a	32.9 (1.4,21) ^a	6.3 (0.6,8) ^a	9.1 (1.5,5) ^b

Table 12. Levels of total lipids, total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides in blood sera of white suckers collected from control (LOK) and metal-contaminated (MAN) lakes during different phases (PRES, SPAW, POST, RECR) of the reproductive cycle. Values are given as means (S.E., n). Within sex, values sharing a superscript are not significantly different. Upper case superscripts are restricted within site. An asterisk designates a significant difference between sites.

Sex	Phase	Total lipids (g l ⁻¹)		Total cholesterol (mg dl ⁻¹)		HDL cholesterol (mg dl ⁻¹)		Triglycerides (mg dl ⁻¹)	
		MAN	LOK	MAN	LOK	MAN	LOK	MAN	LOK
M	PRES	23.8(5.5,6) ^{a,b}	23.0(3.9,6) ^{a,b}	361.2(34.3,5) ^a	347.7(16.9,5) ^a	51.0(5.5,5) ^a	57.0(1.3,4) ^{A,B}	926(107,5) ^a	1383(236,5) ^a
M	SPAW	32.6(2.4,6) ^b	27.5(3.1,6) ^b	381.4(29.9,6) ^a	355.4(14.2,6) ^a	71.9(2.0,5) ^b	63.0(3.8,5) ^{A,B}	454(110,5) ^b	755(190,5) ^b
M	POST	22.9(3.2,6) ^a	20.2(1.6,6) ^a	329.7(17.9,6) ^a	302.7(62.2,6) ^a	62.6(2.9,5) ^{ab}	47.8(4.7,4) ^B	851(185,4) ^{a,b}	605(156,4) ^{ab}
M	RECR	17.0(1.7,6) ^a	17.1(1.4,6) ^a	338.3(33.9,6) ^a	300.3(59.6,6) ^a	62.2(4.6,5) ^{ab}	67.2(3.9,5) ^A	618(176,5) ^b	707(113,4) ^b
F	PRES	16.8(4.3,6) ^a	19.9(2.2,6) ^a	294.7(28.8,6) ^a	255.7(36.6,6) ^A	46.6(4.2,5) ^a	46.0(4.1,5) ^a	548(116,5) ^{a,c}	840(256,5) ^{a,c}
F	SPAW	20.2(2.4,6) ^a	15.6(1.6,6) ^a	308.0(21.0,6) ^a	216.9(28.3,6) ^A	59.4(2.7,5) ^a	56.7(6.1,4) ^a	667(373,4) ^a	254(77,5) ^a
F	POST	18.3(3.4,6) [*]	28.6(3.1,6) [*]	314.2(29.3,6) ^a	257.0(30.1,6) ^A	49.8(4.2,5) ^a	54.0(8.6,5) ^a	1678(294,5) ^{b,c}	1413(250,5) ^{c,b}
F	RECR	22.1(3.3,6) ^a	23.3(1.5,6) ^a	414.0(12.6,6) ^b	381.0(24.3,5) ^B	67.4(9.0,4) ^a	55.0(4.4,5) ^a	894(152,5) ^c	653(143,5) ^c

Figure 7: Liver stores of glycogen and lipids. Values (mg g^{-1}) represent pooled data for MAN and LOK, numerical data are shown in Table 11.

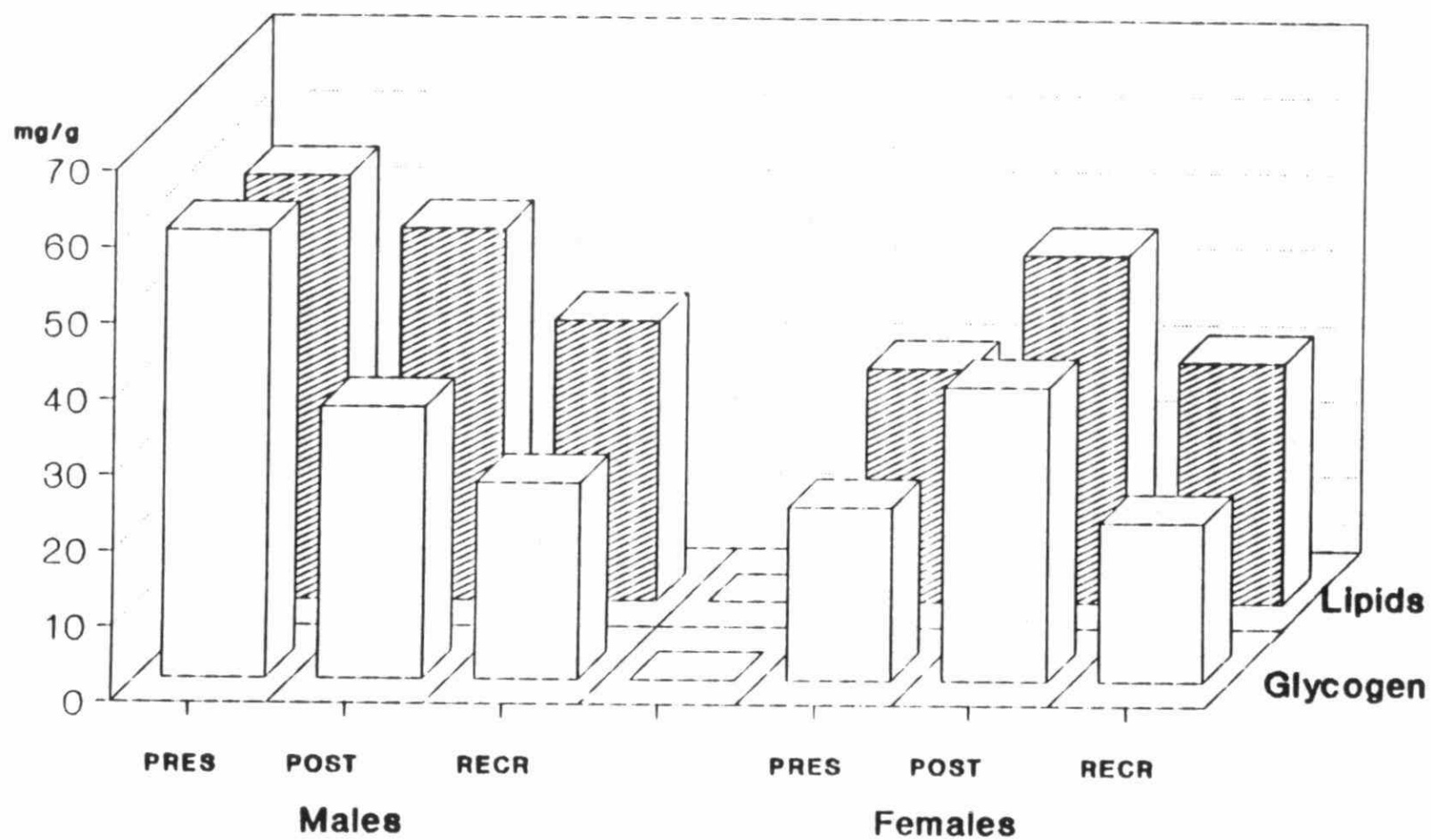
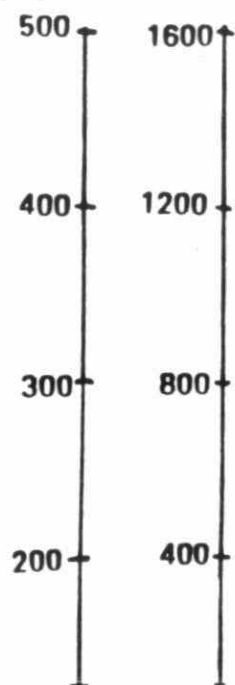


Figure 8: Serum levels of lipids, triglycerides and cholesterol. Levels for lipids (g dl^{-1} ; triangles), total cholesterol (mg dl^{-1} ; open circles) and triglycerides (mg dl^{-1} ; closed circles) are pooled data for MAN and LOK. Total lipids for females are split in POST since MAN (dotted line) was significantly less than LOK.

○
TOTAL
CHOLESTEROL (mg dl⁻¹)

●
TRIGLYCERIDES (mg dl⁻¹)



PRES SPAW POST RECR

MALES

△
TOTAL
LIPIDS (g L⁻¹)



PRES SPAW POST RECR

FEMALES

HDL cholesterol levels exhibited a marginal interaction between sex, site and season ($p=.056$) (Table 12). In MAN males, PRES levels were significantly less than SPAW ($p=.016$) and RECR levels of LOK males were significantly higher than POST ($p=.015$). In females, there was no effect of season ($p=.058$).

There were no significant effects of sex ($p=.78$), site ($p=.78$) or season ($p=.69$) on the levels of total serum protein (Table 13). At LOK, there was no effect of sex ($p=.09$) or season (PRES, SPAW, RECR, $p=.40$) on serum calcium levels. MAN levels of serum calcium were significantly higher than LOK ($p=.02$), and values for females were higher than males ($p=.007$), although there was no seasonal difference between PRES and RECR ($p=.71$) (Table 13). There was no effect of site on alkali-labile protein-bound phosphorus ($p=.17$). There were significant effects of season on serum protein-bound phosphorus ($p=.005$) and SPAW levels were significantly lower than PRES levels.

Table 13. Levels of protein, calcium and alkali-labile protein-bound phosphorus in blood sera of white suckers collected from control (LOK) and metal-contaminated (MAN) lakes during different phases (PRES, SPAW, POST, RECR) of the reproductive cycle. Values are given as means (S.E., n). Values sharing a superscript are not significantly different.

Sex	Phase	Serum protein (mg ml ⁻¹)		Serum Ca (mg dl ⁻¹)		Serum P (mg dl ⁻¹)	
		MAN	LOK	MAN	LOK	MAN	LOK
M	PRES	38.0(2.2,6) ^a	42.3(2.4,6) ^a	17.2(3.1,10) ^c	13.6(4.1,8) ^a	-	-
M	SPAW	41.1(2.7,6) ^a	43.9(4.3,6) ^a	-	15.1(3.5,8) ^a	-	-
M	POST	39.6(0.7,6) ^a	41.2(3.7,6) ^a	-	-	-	-
M	RECR	37.6(1.4,6) ^a	38.2(2.0,6) ^a	11.4(1.6,10) ^c	9.1(0.5,8) ^a	-	-
F	PRES	43.0(3.4,6) ^a	38.6(1.6,6) ^a	20.9(2.6,10) ^b	12.6(2.4,8) ^a	16.6(3.4,8)	16.3(3.7,7)
F	SPAW	41.0(2.3,6) ^a	40.8(3.8,6) ^a	-	16.5(3.2,8) ^a	4.7(1.7,6)	10.8(3.1,7)
F	POST	53.4(2.7,6) ^a	38.8(1.7,6) ^a	-	13.7(3.1,6) ^a	16.1(5.6,5)	24.0(3.4,8)
F	RECR	42.3(3.3,6) ^a	41.1(2.2,6) ^a	16.6(1.5,10) ^b	17.6(1.7,8) ^a	10.2(1.6,8)	8.1(1.8,8)

3.4 Discussion

The lack of significant relationships between weight and age for female fish at contaminated sites suggests that these fish did not show significant somatic growth during the period of examination. Examination of size and age data showed that LOK females were longer and heavier than contaminated fish at all ages examined after sexual maturation (7-9 y). Although exposure to metals has been associated with decreased growth, (Lett et al., 1976; Dixon and Sprague, 1981; Seim et al., 1984; Collvin, 1985), a transient decrease usually occurs at the beginning of exposure (Sinley et al., 1974). This initial decrease in growth has been attributed to the synthesis of protective proteins (Roch et al., 1982; Bradley et al., 1985), and to initial decreases in appetite (Bengtsson, 1974; Lett et al., 1976). Although acclimation of growth may be slow (Collvin, 1985), growth rates return to normal (Buckley et al., 1982) and eventually may surpass the growth of controls (Pickering, 1968; McKim and Benoit, 1971; McFarlane and Franzin, 1978), possibly due to increased food intake (Farmer et al., 1979). These changes are not consistent with those reported in this study. In this study, the growth differences at contaminated sites became more apparent as the fish aged. The effect on females was greater than males, and the correlation between decreased size and sexual maturation of females at the contaminated sites suggests that the fish were unable to meet the energetic demands of both growth and reproduction after sexual maturity. Females at the contaminated sites exhibited decreased serum lipid levels during the postspawning period and decreased muscle lipid levels during the period of gonadal growth. Males did not exhibit significant differences in these characteristics. Visual observation of visceral lipid deposits before winter were also suggestive of decreased energy stores

at the contaminated sites. Lipid levels in suckers have been found to decline over the winter period (Lalancette, 1976), and the lower deposits in these fish are suggestive of decreased energetic reserves during gonadal growth. Again the effect on females was greater than males, and females attribute a higher proportion of their energetic stores to reproduction.

A large proportion of young females failed to release their eggs at the contaminated sites. It may not be unusual to find females with mature ovaries after the completion of the normal spawning period; they have been recorded in Lake Superior (Portt, pers. comm.), in other studies (Lalancette, 1973), and the phenomenon was evident at the reference site in this study. Consequently, the effects can not be attributed to the effects of chronic metal exposure alone. The young age of the fish which failed to spawn at the LOK suggests that they were attempting to spawn for the first time. Spawning failures at the contaminated site did not appear to be restricted to first-time spawners. Failure to release eggs has been found in white suckers exposed to a mixture of zinc, copper and cadmium, but was not evident at the reference site and no mention was made of an age-associated effect (McFarlane and Franzin, 1978). It does not appear that this phenomenon is related to the apparent two year spawning cycle in some northern fishes (Dutil, 1986) since the fish had fully matured ovaries at the time of failure.

Although fecundity usually increases with fish length (Wootton, 1979), fecundity at the contaminated sites exhibited no relationship with age and is a reflection of the failure of these fish to exhibit significant increases in size after sexual maturity. McFarlane and Franzin (1978) reported an increased fecundity in metal-exposed suckers, but metal exposure has been associated with a decreased brood size and lar-

val size in guppies (*Poecilia reticulata*) (Uviovo and Beatty, 1979) and a decreased fecundity in fathead minnows (*Pimephales promelas*) (Brungs, 1969). Fecundity and egg size was significantly decreased at the contaminated sites, suggesting that less energy was committed to reproduction at those sites. Changes in contaminated fish in this study appear to be related to a decreased food availability.

Both the quantity and quality of food can affect bioenergetics, the amount of energy for growth (Dixon and Hilton, 1981) and the chronic toxicity of metals (Dixon and Hilton, 1985). Furthermore, there is a direct association of food supply with both the growth of suckers (Tripple and Harvey, 1987a) and fecundity in salmonids (Scott, 1962; Bagenal, 1969). When food consumption levels are unfavourable, egg production takes place at the expense of decreased weight and energy content of soma in some species (Hislop et al., 1978; Wootton, 1979). Although food restriction and age of maturity can affect both fecundity and growth, suckers at the contaminated sites did not show significant differences from fish from the reference sites until sexual maturation. This suggests that the nutritional deficiency is marginal and that the fish can grow normally until faced with the added demands of reproduction.

The increased liver size at the contaminated sites is consistent with increased metabolic activity (Larsson et al., 1985). Since the fish were capable of achieving normal liver glycogen and lipid levels, and since the average size of fish was not different until after maturation, any hepatic impairment appears to be negligible. Liver metal burdens were elevated at the contaminated sites, as were the levels in kidney, gills and ovaries (Miller et al., 1988), but again metabolic impairments were not obvious. The decreased prevalence and intensity of parasitic infections has been

previously reported for suckers collected from contaminated sites (Swanson, 1982). It is unknown whether the decreased success of parasites in contaminated environments is associated with a decreased tolerance of the adults or the intermediate stages.

Serum protein, total lipids and serum triglyceride levels were within the range of values previously reported for suckers (Hille, 1982; Lockhart and Metner, 1984). Although changes in serum protein and total lipids were not suggestive of increased production during vitellogenesis, levels of triglycerides and total cholesterol were. Furthermore, alkali-labile protein-bound phosphorus levels in the serum were significantly higher during the prespawning period than during spawning. Serum calcium levels in female fish were elevated over males during RECR at both sites. These changes appeared to be related more to a decline in male calcium levels than to an increase in females. Seasonal changes in female serum calcium were not significant at the reference site. In white suckers, the failure to release eggs as a result of acidification has been related to decreased serum calcium levels (Beamish et al., 1975), although the calcium levels in both female and male fish, and the ratio between the sexes were similar to those observed in this study during successful reproduction of suckers. Measurements of serum calcium, protein and phosphoprotein have been used as crude indicators of vitellogenesis (Nagler et al., 1987), and have been shown to follow reproductive changes in salmonids (Hille, 1982; Duston and Bromage, 1986). It is apparent that catostomids may not exhibit "normal" (salmonid) seasonal changes in blood parameters during gonadal development.

White suckers collected at MAN and LMN exhibited decreased growth after sexual maturity, an increased incidence of spawning failure and a decreased fecundity

and egg size. These changes are all suggestive of a decrease in the availability of nutrients at the contaminated sites.

Chapter IV

LARVAL SURVIVAL, GROWTH AND TOLERANCE TO COPPER

4.1 Introduction

Aquatic organisms from metal contaminated sites commonly show increased tolerance and/or resistance to those metals on subsequent exposure (LeBlanc, 1982; Duncan and Klaverkamp, 1983; Benson and Birge, 1985). The response is, however, inconsistent; Rahel (1981) reported no increase in the zinc tolerance of common shiners (Notropis cornutus) from zinc-contaminated sites. While physiological adaptation (acclimation) to metals can develop rapidly (Dixon and Sprague, 1981), the onset of genetic adaptation appears to be slow. Rahel (1981) found that although flagfish (Jordanella floridae) exhibited increased zinc tolerance after acclimation, three generations of selection did not increase the response. Furthermore, McKim (1977) found that in over half of the life cycle tests which he reviewed there was no difference in toxicant response between first and second generation fish larvae.

Metal contamination has consistently been implicated in the reproductive failure of fish populations. Copper mining wastes were thought to be associated with neoplasms and reproductive failure of sauger (Stizostedion canadense) in Torch Lake, Michigan (Black et al., 1982). Copper has been shown to reduce the survival of bluegill (Lepomis macrochirus) larvae at concentrations of 40 to 162 $\mu\text{g l}^{-1}$ (Benoit, 1975) and the hatchability of brook char (Salvelinus fontinalis) eggs is reduced by copper concentrations as low as 9.4 $\mu\text{g l}^{-1}$ (McKim and Benoit, 1974).

This study was initiated to examine

- the growth, development and survival of larvae from contaminated lakes
- the larvae for possible genetic adaptation with respect to their response to metal exposure.

4.2 Methods

White suckers were collected from the spawning streams on each lake by dipnet in early May, 1986. Males were anesthetized with tricaine methanesulfonate (MS-222) and dried with paper toweling. After a 10 ml borosilicate pipette was inserted into the vent of the fish, gentle pressure was applied to the pectoral region, and the volume of milt expressed was measured to the nearest 0.5 ml. The milt was stored on ice in 100 ml closed polyethylene cups; subsamples were collected in unheparinized hematocrit tubes. Spermatocrit estimates (percent packed sperm cells) were recorded after spinning at maximum speed on an International Clinical Centrifuge (Model CL) for 7 min. Females were anesthetized, dried and eggs were expressed into 100 ml polyethylene cups and stored on ice. Gametes were handled and transported as quickly as possible, and all fertilizations were performed within 4 h of collection.

4.2.1 Preliminary trials

In order to test the method for fertilization tests, preliminary assays were performed on suckers from Lake Ontario. Six females and eight males were collected from Shelburne Creek, near Oakville, Ontario, (Lake Ontario) when the water temperature was 10°C. The fish were transferred to 12°C water at the laboratory at the University of Waterloo, where ovulation occurred after 2 d. Eggs were collected from the

same five females at 8:00 and 20:00 and fertilized with subsamples of the pooled milt collected from three males. In addition, the 8:00 samples were stored after collection in 100 ml linear polyethylene cups on ice, and were fertilized at 0, 1, 2 and 4 h after collection. All fertilizations were performed in duplicate. The fertilized eggs were incubated in sieves at 10.5°C and exposed to a 1 h flow-through malachite green treatment (2.0 mg l⁻¹) on alternate days. Fertilization estimates were performed at 2, 4 and 6 d post-fertilization.

4.2.2 Field trials

At both the contaminated site (MAN) and the control site (LOK), 9 females and 10 males were collected by dipnet from the spawning beds. Since the females collected at the LOK site were not ripe during the first trial, females were collected from an adjacent uncontaminated headwater lake (Straight Lake, Figure 1). Two sets of fertilizations were performed in duplicate at each site. Subsamples of eggs from females were pooled and fertilized with milt from individual males, and a pool of 5 to 7 males was crossed with each female. The eggs were fertilized in plastic weigh boats, water hardened with clean water from the control site, and transferred to incubation cups (100 ml polyethylene beakers, modified by replacing the bottom with #656 Nyltex screening). The cups were placed in clean water in a 40 l insulated cooler for transport to the University of Waterloo laboratory 4 d later. The water temperature remained at 8°C (MAN) or 8 to 11°C (LOK) during this period. At Waterloo, the eggs were transferred to 11°C water and incubated until hatching. The eggs were treated every second day with malachite green as above, and fertilization estimates were performed at 6 d post-fertilization. Deformity estimates were performed at hatch.

4.2.3 Cross-fertilizations and field collections

Milt was collected from 14 males at LMN at 10:00. In addition, 11 males and 10 females were stripped at LOK at 11:30, the eggs were pooled at 13:00 and fertilized with the individual milt of at least 10 males from each site in duplicate. In addition, to get an estimate of natural fertilization performance, 25 to 100 eggs were collected from 10 different spawning beds in Agam Creek (MAN; Figure 1). At the control site eggs were much harder to locate, so eggs from 10 different locations in the stream were collected and pooled (156 eggs). All eggs were returned to the University of Waterloo for examination.

4.2.4 Incubations and larval rearing

Eggs were incubated in 64 l polyethylene tubs receiving 1 l min⁻¹ of well water. Incubations occurred in either 100 ml fertilization cups or linear polyethylene strainers. All larvae were checked for deformities at hatch, and then released to the bottom of the 64 l rearing chamber at 11 to 12.5°C. There was some asynchrony in hatching between incubation chambers. Eggs incubated in the cups did not hatch until the eyes of the larvae had fully pigmented. Eggs in the strainers hatched before the eyes had begun pigmentation. Similar asynchrony has been previously reported and the hatch of larvae with pigmented eyes was interpreted as the actual hatch date (McElman and Balon, 1980). At 5 d post-hatch the larvae were separated into two groups. One group from each lake received no food (starved) and the other group was given food starting on day 5. A variety of foods (minced boiled lettuce, frozen or live brine shrimp, minced tetra-min) was offered three to four times daily. No feeding was observed before 18 d after hatching.

Fish were checked daily for developmental changes and samples were preserved in 10% buffered formalin 4, 12, 16, 20, 21, 25, 29, 33 and 37 d after hatching. After preservation the larvae were measured for length (0.5 mm) and total body weight (1.0 mg). Leslie and Moore (1986) concluded that white sucker larvae did not show significant changes in length after preservation in formalin. After the total weight was estimated, the yolk reserves were carefully dissected away and body weight was estimated (1.0 mg). The relative contribution of the yolk to the total weight was estimated from these calculations.

4.2.5 Bioassays

All 64 l tanks received aerated well water at 1 l min⁻¹. Hardness, alkalinity and pH were measured weekly from randomly selected tanks. Mean (SE) characteristics of the test water were: temperature, 13.6 (0.2)^oC; total hardness, 332 (6.0) mg l⁻¹ as CaCO₃; Ca hardness, 231 (7.0) mg l⁻¹ as CaCO₃; Mg hardness, 96 (3.0) mg l⁻¹ as CaCO₃; and alkalinity, 257 (6.0) mg l⁻¹ as CaCO₃. Experimental photoperiod was 16 h light:8 h dark, with 0.5 h of gradual dawn and dusk included in the light portion.

Bioassays were conducted on the suckers at ages (d post-hatch) of 1 d (LOK unfed only), 5 d (LOK and MAN unfed only), 9,13,17,21,25 and 29 d (LOK and MAN, fed and unfed), 33 d (LOK only) and 37 d (LOK and MAN, fed only). Bioassays on fed crossfertilized larvae were conducted at 9 d, 33 d and 37 d. In addition, Lake Ontario and MAN suckers were tested at 3 months and 6 months, and LOK fish were tested at 6 months of age. Due to the differences in spawning time, bioassays on similarly aged LOK and MAN larvae were started 4 d apart. The fish were not fed during the 144-h exposures. Copper stock solution (prepared

from C.P. grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was dispensed into the dilution water streams from mariotte bottles (Leduc, 1966). Water samples, acidified to 1% with nitric acid, were taken from each tank daily and stored in linear polyethylene bottles for copper analysis by flame atomic absorption spectrophotometry.

For each bioassay, ten white sucker larvae from each treatment were placed in nominal concentrations of 0, 150, 300, 450, 600, 900, 1200 and 2200 $\mu\text{g Cu l}^{-1}$. Mean assayed copper concentrations (SE,n) were 3.1 (2.7,54), 167 (12,52), 271 (64,46), 360 (32,52), 610 (54, 52), 944 (75,51), 1098 (86, 52) and 2258 (251,48) $\mu\text{g l}^{-1}$. Suckers tested at 1 (LOK), 5 (LOK and MAN) and 9 d (MAN only) post-hatch were not exposed to the upper two concentrations. Suckers younger than 3 months of age were tested in 100 ml polypropylene cups, modified by replacing the base with Nytex screening and adding a styrofoam floatation collar. Older suckers were tested in linear polyethylene strainers (diameter, 12 cm; depth, 2.5 cm; volume, 240 ml). In all cases the test chambers were floated in the bioassay tanks described above. The 96-h LC50s and LT50s were calculated using probit analysis (Finney, 1971). No control mortalities were evident in bioassays initiated prior to 17 d post-hatch. At 21, 25, 29 and 33 d, however, control mortalities were excessive. As such, LT50s were calculated using only results for concentrations from 900 to 2200 $\mu\text{g Cu l}^{-1}$. Control mortalities did not occur during the period of resistance at these upper concentrations.

4.2.6 Statistics

Fertilization data were transformed with an arcsine square root function, and fertilization and growth data were compared using analysis of variance. When differences were significant ($p < 0.05$), treatment effects were examined using Tukey-Kramer's test (Zar, 1984). The preliminary fertilization trials and larval size data were compared using t-tests. Lethality data was compared according to the methods described by Sprague and Fogels (1977).

4.3 Results

There was no effect of time of day on the fertilization rate of eggs collected during am (8:00) or pm (20:00) time periods from the same fish ($p = 0.275$). There was also no detectable decrease in fertilization rate of gametes stored on ice for 4 h. Eggs fertilized immediately after collection yielded a fertilization rate of 89.4% (SE, 2.1; n, 10) while subsamples of the gametes fertilized 4 h later yielded a fertilization rate of 92.2% (1.3, 10). Estimates of fertilization rate after 4 d incubation were significantly lower than estimates taken after 2 d incubation ($p = 0.008$), but estimates after 6 d incubation were not different from 4 d ($p = 0.052$). At 6 d post-fertilization, larvae were visible within the egg, and all subsequent fertilization estimates were made at 6 d.

There was no significant difference in fertilization rate between MAN and control females ($p = 0.590$) (Table 14). Individual males were used to fertilize pools of eggs at each site and MAN estimates were lower than LOK ($p = .019$), but identical trials using milt from LMN found the opposite to be true. Cross-fertilization of LOK eggs with LMN milt yielded higher fertilization rates than fertilization of the identical batch of eggs with LOK milt (Table 14) ($p < .0001$).

Table 14. Fertilization rate of eggs collected at the sites, water hardened in clean water and returned to Waterloo for incubation.

Site	Sex	Fertilization rate (%)
MAN	Female ¹	79.7±5.5 (20) ²
	Male ³	87.7±2.3 (20)
LOK	Female	82.2±1.7 (20) ⁴
	Male	92.8±0.8 (20)
<u>Cross-fertilized</u>		
LMN male x LOK female		83.7±3.3 (24)
LOK male x LOK female		67.2±1.8 (20)

¹Individual females crossed with pool of males

²MAN redd collections 1985 64.9±11.5 (9)
1986 78.3±2.2 (10)

³Individual males crossed with pool of females

⁴LOK redd collection 1986 78.0

While naturally fertilized eggs were plentiful at the MAN spawning sites, few eggs were available at the control (LOK) sites and those present were highly fungused. Since sufficiently large egg samples were unavailable from individual redds at the control site, a pooled sample of 156 eggs from 10 redds was taken. The mean (SE) fertilization rate of eggs collected from 10 redds at MAN was 78.3% (7.6) while the fertilization rate of the pooled LOK sample was 78.0%. Prehatch weight of eggs was significantly higher at LOK than at MAN (Table 15; $p < 0.001$), and the weight of eggs from both sites was significantly larger than naturally fertilized eggs collected from the MAN redds ($p < .001$).

The milt volumes of male suckers were significantly higher at either MAN or LMN than at either LOK or Lake Ontario sites ($p = 0.009$; Table 15), but there was no difference in the concentration of sperm cells (spermatocrit; $p = 0.672$). There was no effect of milt volume or sperm concentration on the fertilization rate using either LMN milt ($p = 0.17, 0.07$) or LOK milt ($p = 0.90, 0.35$).

MAN larvae were significantly shorter ($p = 0.008$) and lighter ($p = 0.011$) than the LOK larvae at 2 d post-hatch (Table 15). The incidence of gross deformities was higher for eggs collected from MAN redds than for eggs from MAN or LOK hatched in the lab (Table 16). A "c" shaped deformity was predominant at the time of hatching, representing 100% of the LOK deformities, 82% of the MAN lab and 87% of the MAN redd deformities. Only 29% of deformities found in Lake Ontario suckers at the time of hatching were of this type (Table 16).

Both MAN and LOK larvae developed at rates comparable to published developmental times (McElman and Balon, 1980). Although MAN larvae reached crucial developmental stages at times comparable to LOK larvae, the occurrence of swim

Table 15. Characteristics of the milt, eggs and larvae from the study lakes. Values are shown with the mean \pm s.e. (n).

Site	Milt		Egg	Larval	
	Volume (ml)	Spermatocrit (%)	Weight (mg/10 eggs)	Length (mm)	Weight (mg)
MAN	2.39±0.62 (9)	---	187±3 (20) ¹	10.48±.10 (30)	6.6±0.2 (30)
LMN	3.25±0.62 (14)	44.6±5.3 (14)	---	---	---
LOK	1.36±0.14 (21)	47.5±5.0 (10)	172±3 (20)	10.85±.12 (30)	7.3±0.3 (30)
LOnt	1.75±0.42 (6)	39.7±3.9 (6)	---	---	---

¹MAN redd, egg size 124 \pm 4 (10)

Table 16. Types of deformities seen in suckers eggs. During the 1986 collections LOK eggs exhibited a deformity frequency of 1.5% (13/867), while MAN eggs were 2.2% (28/1257). Eggs collected from the MAN redds in 1985 exhibited a frequency of 6.0% (94/1544).

<u>Deformity type and % of deformed larvae exhibiting abnormality</u>						
	Corkscrew	L-shaped	C-shaped	Yolk at posterior	Two-headed	Short body
MAN ¹	-	3	87 ²	9	-	1
LOnt ³	48	7	29	14	2	-

¹MAN redd collections

²Eggs from MAN incubated at the laboratory exhibited 82% deformities of this type, while LOK larvae exhibited 100% of this type. All deformed larvae which survived hatching in 1985 were dead within 14 d of hatching.

³Gametes were collected from fish held for two weeks in the laboratory after spawning. Deformities among larvae from fresh gametes are usually very rare (<2%).

MAN eggs collected in 1987 and incubated in the LOK spawning stream exhibited a deformity rate of 4.8% (17/351), while those incubated in the laboratory exhibited a frequency of 5.0% (26/520). MAN eggs incubated at the MAN site exhibited a deformity rate of 10.0% (33/331).

bladder inflation, first feeding and completion of yolk utilization occurred slightly earlier (Table 17). Size data for fed and unfed larvae exhibited no difference before first feeding (18 to 20 d post-hatch), and data were pooled. From 4 to 20 d post-hatch, there was a significant effect of age on total weight ($p < 0.0001$), but no effect of site ($p = 0.80$, Figure 9). Body weight, however, showed a significant interaction between site and age ($p = 0.003$). Although there was no difference in body weight at 4 d post-hatch MAN larvae exhibited a slower increase in body weight, and had used all of their yolk reserves by 20 d post-hatch (Table 18). Between 21 and 29 d post-hatch, LOK larvae were significantly larger than MAN larvae ($p = 0.01$), and there was an interaction between age and feeding status ($p = 0.003$).

MAN larvae also died at an increased rate, and all unfed larvae were dead by 29 d post-hatch (Figure 10), while some LOK larvae survived without food until 42 d post-hatch. Furthermore, an increase in the survival rate of MAN fed larvae over unfed could be detected by 25 d post-hatch, while a similar point for LOK was 34 d (Figure 10). At death from starvation, MAN (29 d) and LOK larvae (39 d) were not significantly different in weight (Table 18). MAN larvae showed a much poorer conversion to feeding, and failed to show significant increases in weight before 37 d post-hatch (16 d post-feeding), although LOK fed larvae exhibited consistent weight increases after first feeding. Between 33 and 37 d post-hatch, there was a significant effect of both site ($p = 0.0001$) and age ($p = 0.003$) on larval weight. This was also reflected in the survival of fed larvae (Figure 10).

Bioassays were run on hatched larvae beginning at 1 d post-hatch and continuing every 4 d until 37 d. Preliminary bioassays on 6 month old suckers found the 96-h LC50 to be 422 ug Cu l^{-1} (95% fiducial limits: 364 to 455 ug l^{-1}) and the sur-

Table 17. Age at which white sucker larvae from control (LOK) and contaminated (MAN) sites reached crucial developmental stages (McElman and Balon, 1980) at 12°C. Times are given in days post-hatch and daily temperature units (DTU).

Critical Stage	MAN site			LOK site		
	Age (d)	DTU (°d)	Incidence (%)	Age (d)	DTU (°d)	Incidence (%)
I. First swimming (<2.5 cm)	5	60		5	60	
II. Bile in gall bladder, gill circulation complete.	7	84		7	84	
III. Mouth opening and closing, pectoral fins moving and blood entering gill arches	9	108		9	108	
IV. Swim bladder inflated	9	108	30	10	120	5
	11	132	90	12	144	50
	12	144	100	13	156	100
V. First feeding, first feces	18	216	20	20	240	20
	21	252	100	22	264	100
VI. Yolk absorption complete	18-20	196-204		20	204	

Figure 9: Growth of larvae after hatching. Larvae from MAN (open symbols) and LOK (shaded symbols) are shown up until the time of first feeding (20 d) and up to 37 d post-hatch. Data are shown in Table 18.

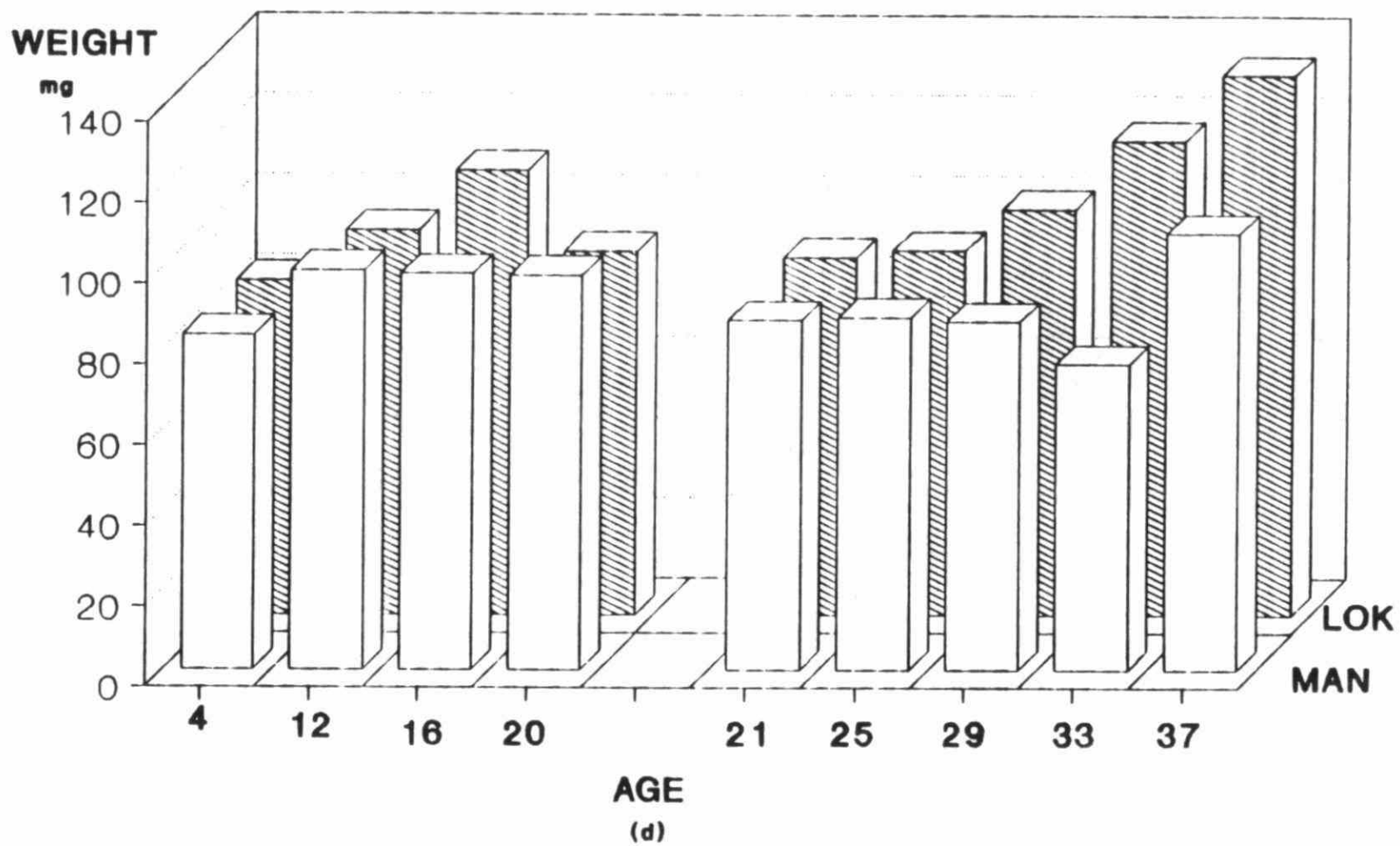


Table 18. Total wet weight, body weight (total weight-yolk weight) and percent yolk of white sucker larvae at different ages post-hatch. Values are given as mean \pm s.e.(n).

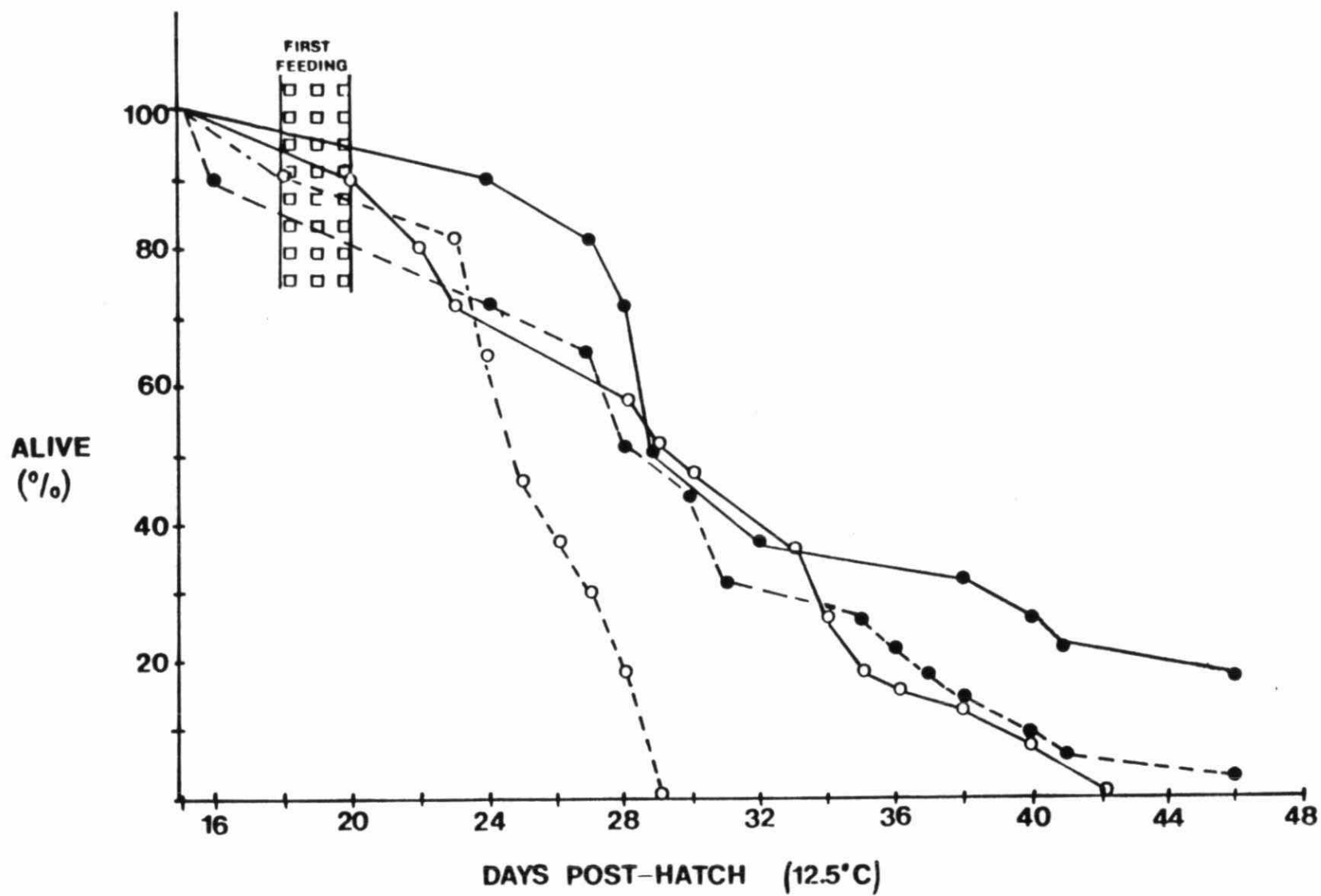
<u>A. During yolk utilization</u>						
Age (d)	LOK			MAN		
	Total Weight (mg)	Body Weight (mg)	Proportion of yolk (%)	Total Weight (mg)	Body Weight (mg)	Proportion of yolk (%)
4	82.9 \pm 2.7 (20)	51.2 \pm 2.3 (20)	38.2	83.0 \pm 2.3 (10)	51.7 \pm 1.2 (10)	37.7
12	95.4 \pm 2.9 (5)	81.0 \pm 2.0 (5)	15.1	99.2 \pm 3.5 (17)	62.2 \pm 3.8 (17)	37.3
16	110.0 \pm 4.7 (10)	95.1 \pm 7.0 (10)	13.5	98.3 \pm 3.0 (20)	80.1 \pm 3.7 (20)	18.5
20	90.3 \pm 3.4 (15)	86.9 \pm 4.6 (15)	3.8	98.0 \pm 8.5 (10)	98.0 \pm 8.5 (10)	0

<u>B. Total Weights (mg) after first feeding</u>				
Age (d)	LOK		MAN	
	Fed	Unfed	Fed	Unfed
21	89.0 \pm 4.1 (15)	96.0 \pm 4.9 (10)	86.9 \pm 3.3 (15)	89.7 \pm 3.5 (10)
25	90.7 \pm 2.7 (25)	90.0 \pm 4.6 (10)	87.8 \pm 3.4 (20)	87.0 \pm 2.4 (20)
29	101.0 \pm 5.6 (10)	81.8 \pm 3.4 (10)	86.7 \pm 3.4 (20)	75.0 \pm 4.5 (6)
33	118.0 \pm 11.4 (10)	71.5 \pm 4.9 (10)	76.2 \pm 4.5 (15)	1
37	134.4 \pm 6.3 (10)	76.0 \pm 4.0 (10)	108.8 \pm 7.6 (10)	1

¹Complete mortality.

Figure 10:

Survival of larvae after hatching. Larvae from MAN (open symbols) and LOK (closed symbols) were divided into fed (solid line) and unfed (dotted line) groups at day 5. No feeding was observed before 18 d post-hatch and no mortalities were recorded before 16 d.



vival times at 900 ug l^{-1} to be less than 48 h (unpubl. data). Lethal toxicity did not occur at these concentrations in the larval bioassays until 9 d post-hatch. Two higher concentrations were added after the completion of the first bioassay (1200 and $2200 \text{ ug Cu l}^{-1}$) and survival of the larvae at the highest concentration showed a two-fold increase in the resistance of MAN larvae at 13 d post-hatch (Figure 11). At 9 and 13 d post-hatch it was not possible to calculate an accurate LC_{50} for the MAN larvae since 50% mortality occurred at only one concentration (2200 ug l^{-1}), but LOK larvae showed higher mortality at 1200 , 900 and 600 ug l^{-1} (Figure 12). The 96 h LC_{50} values for combined larvae (fed and unfed) decreased with age at both sites (Table 19). MAN survival times were longer than comparable LOK fish for at similar feeding status at all ages except 21 d, and ages greater than 25 d (Table 20).

Cross-fertilized larvae, formed by the crossing of sperm from contaminated lakes and eggs from the control site, exhibited a $2200 \text{ ug Cu l}^{-1}$ LT_{50} of 18.5 h (fiducial limits, 17.2 to 19.5) at 9 d post-hatch (unfed larvae). This value was not significantly different from the 9 d unfed control (LOK) value of 19.4 h (17.6 to 21.4), but was significantly lower than the comparable values for contaminated eggs (MAN) crossed with contaminated sperm (44.8 and 38.4 h, fed and unfed). Furthermore, the mortality of 9 d cross-fertilized larvae (LOK eggs x MAN milt) at 900 ug l^{-1} was also identical to the control value (30%). At 33 d post-hatch mortality of MAN suckers at 8 and 12 h after exposure to $2200 \text{ ug Cu l}^{-1}$ was 0 and 80 %, and was not significantly different from LOK (0,80), cross-fertilized (0,90) or Lake Ontario suckers (20,80). No differences were detected between Lake Ontario, MAN or LOK suckers at 6 months of age. At 600 ug Cu l^{-1} , the 144 h mortalities

Figure 11: Differences in resistance times (LT50s) of larvae with age. Values are given for MAN (broken lines) and LOK (solid lines) larvae from both fed (closed) and unfed (open) groups after exposure to 2200 ug l⁻¹ Cu. Control mortalities did not occur during the periods of resistance. Values are given with their 95% fiducial limits.

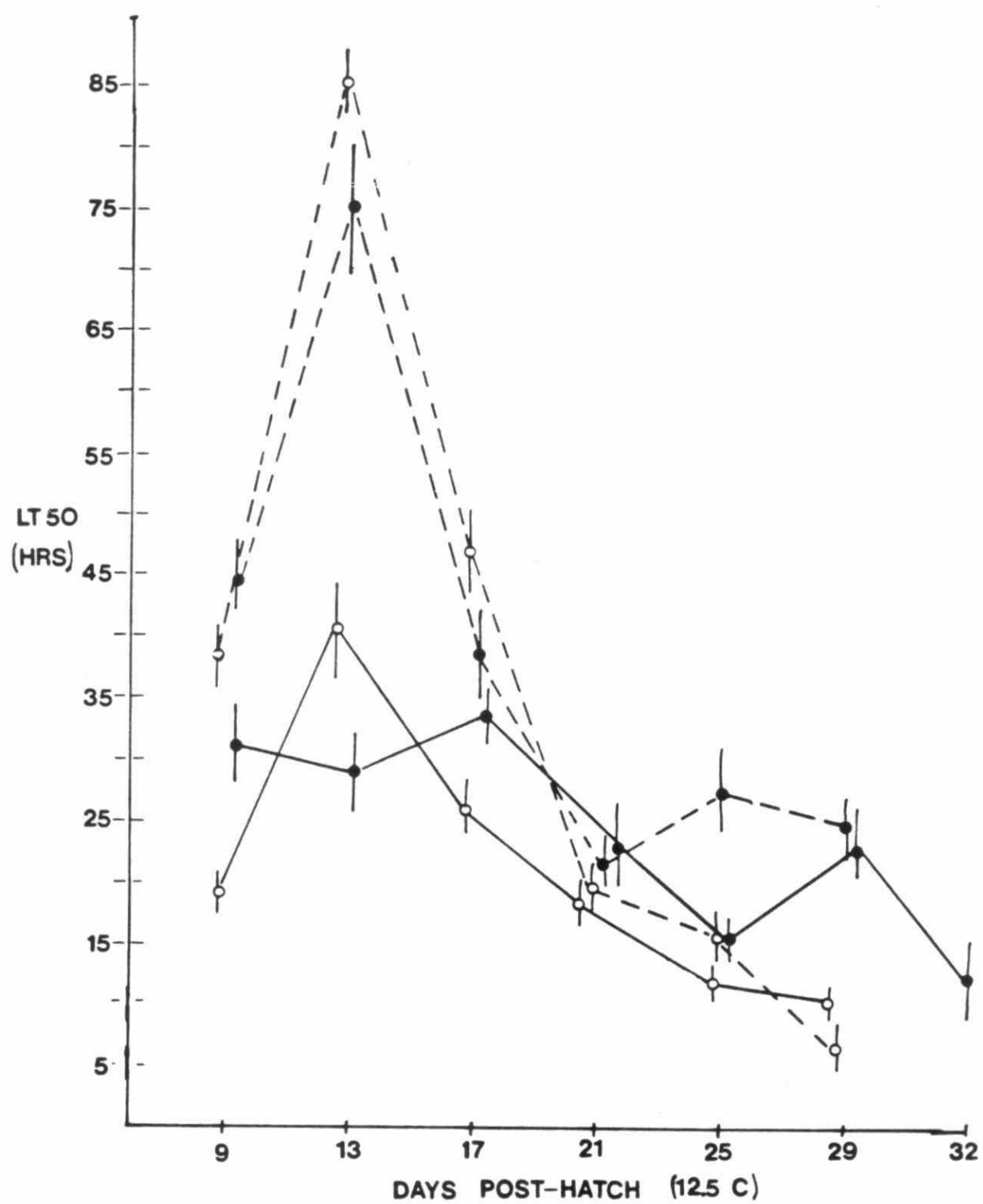


Figure 12:

Mortality data (LC50) after 144 hr exposures to copper. Data is given for larvae (pooled fed and unfed) from MAN (broken line) and LOK (solid line) after exposure to 1200 $\mu\text{g l}^{-1}$ Cu (squares), 900 $\mu\text{g l}^{-1}$ (circles) and 600 $\mu\text{g l}^{-1}$ (triangles). Control mortalities are represented by diamonds. Sample size was 20 larvae and age represents d post-hatch at the start of the bioassay.

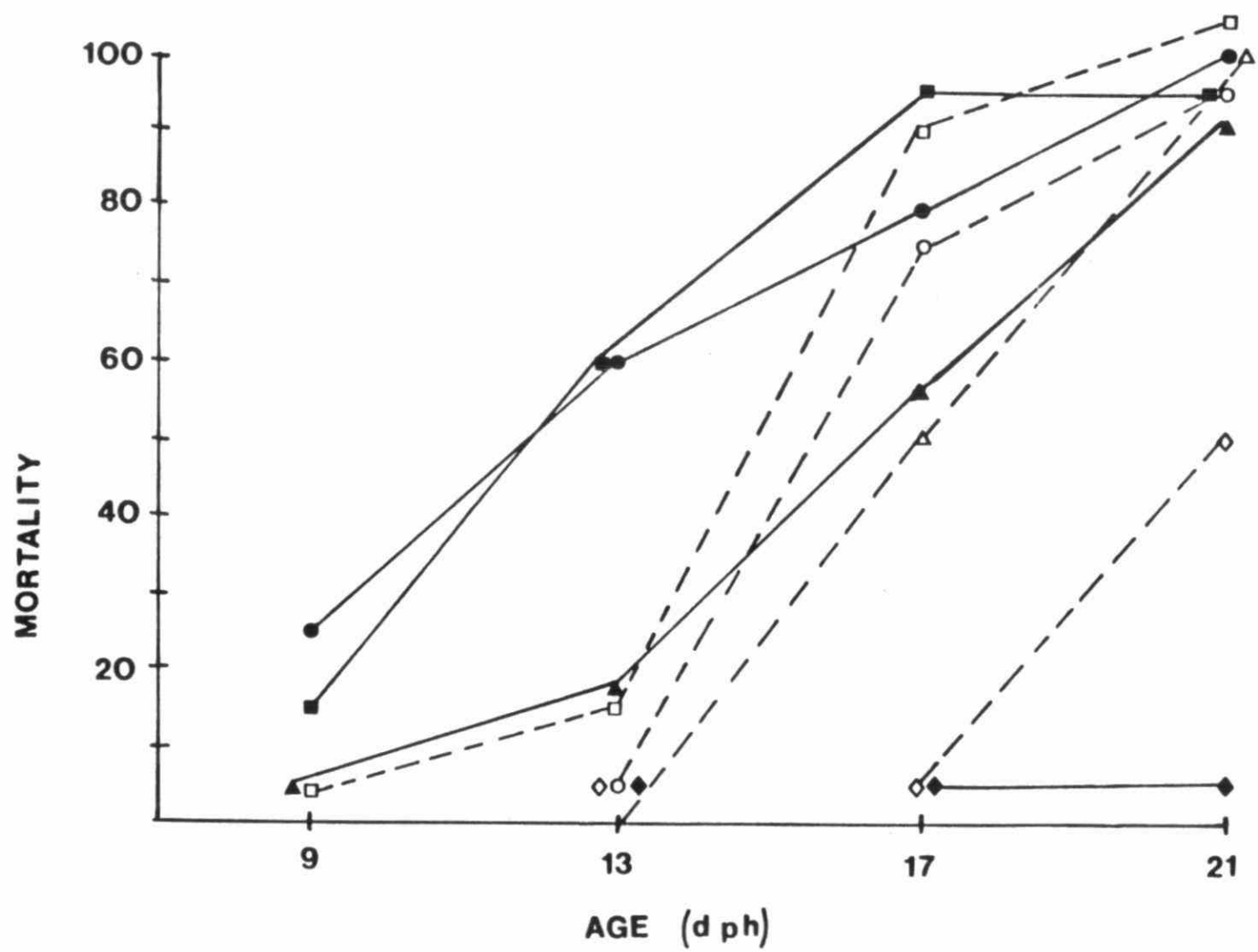


Table 19. Pooled lethality data for fed and unfed white sucker larvae. The results are presented as 96 hr LC50s with the 95% fiducial limits in parentheses. MAN (contaminated site) values at 17 and 21 d are significantly different ($p \leq .05$) from LOK (control site) values.

Age (d)	96 hr LC50 ($\mu\text{g l}^{-1}$)	
	LOK	MAN
13	1273 (1157-1400)	>1200 ¹
17	1051 (998-1107)	1140 (1076-1206)
21	826 (772-884)	653 (570-750)

¹No estimate possible. Complete survival at all concentrations except 2200 $\mu\text{g l}^{-1}$, which showed complete mortality.

Table 20. The effects of age (d post-hatch), site (control, LOK; contaminated, MAN) and ration on the time to 50% mortality (LT50) of larval white sucker exposed to 2200 ug Cu L⁻¹. The 95% fiducial limits for each LT50 are given in parentheses. LOK LT50s with an asterisk are significantly different from the comparative MAN LT50 ($p \leq .05$). Within columns, LT50s without an alphabetical superscript in common are significantly different.

Age (d)	Ration	LOK LT50 (hrs)	MAN LT50 (hrs)
9	fed	31 (29-34)* ^{ab}	45 (42-48) ^a
	unfed ¹	19 (18-21)* ^c	38 (36-41) ^b
13	fed	29 (26-33)* ^{ad}	75 (69-82) ^c
	unfed	41 (37-45)* ^e	85 (82-88) ^d
17	fed	34 (32-36)* ^a	38 (35-43) ^b
	unfed	26 (24-28)* ^{df}	47 (43-50) ^a
21	fed	23 (20-27) ^f	22 (20-24) ^e
	unfed	18 (17-20) ^c	19 (17-22) ^e
25	fed	15 (14-17)* ^g	28 (24-31) ^f
	unfed	12 (11-14)* ^h	16 (14-18) ^g
29	fed	23 (21-26) ^f	25 (22-27) ^f
	unfed	11 ²	7 (5-19) ^h

¹no feeding was detected before 18-20 d post-hatch

²Estimate only, no calculation of fiducial limits possible, not included in analysis.

for 6 month old suckers were 80, 80 and 90% respectively, and all LT50s at 2200 ug Cu l⁻¹ were around 24 h. The 96-h LC50s for 6 month old suckers were 416 ug l⁻¹ (378 to 455) for MAN larvae and 423 ug l⁻¹ (364 to 455) for the controls.

4.4 Discussion

No impacts on fertilization rate were detected in this study either in vitro or in situ. Although metals other than copper can affect the fertilization rate, low copper concentrations have been reported as having no effect in fish (Shaw and Brown, 1971; Billard and Roubaud 1985). Reproduction is, however, usually one of the most sensitive periods of a fish's life history (Donaldson and Scherer, 1983; Woltering, 1984). Effects of various contaminants on reproductive success have been found at concentrations lower than those influencing other developmental stages, and gamete fertility has been proposed as a test criterion (Landner et al., 1985). Reproduction can be altered by copper concentrations in the range of 9.5 to 17 ug l⁻¹ (McKim and Benoit, 1974). Furthermore, zinc is thought to affect egg fragility at concentrations as low as 145 ug l⁻¹, and to promote larval deformities at 295 ug l⁻¹ in soft water (Benoit and Holcombe, 1978). Although water hardness at the Manitowadge site is moderate (100 mg l⁻¹), monthly mean water concentrations can vary from <2 to 34 ug Cu l⁻¹ and from 170 to 650 ug Zn l⁻¹ (Hollinger, pers. comm.). The absence of impairment of fertilization rate and gamete quality is surprising.

MAN larvae exhibited decreased growth, increased developmental rate, poorer survival, a decrease in survival time without food and poorer conversion to feeding, relative to controls. The increased developmental rate of MAN larvae may be related to their decreased size at hatch and an increased developmental rate has been

reported for larvae exposed to zinc (Somasundaram et al., 1984). It is unknown if the 4.5% decrease in size of MAN larvae at hatch is sufficient to account for a 30% decrease in the survival time of unfed larvae. It is possible that the synthesis of protective proteins related to endogenous contaminant residues may represent an energy drain. Although a decline in growth rate is common during acclimatory adaptations to metals, the decline is usually transitory, and conclusions are confounded by sublethal effects, appetite and variations in food intake (Dixon and Sprague, 1981). These factors would not be considered during endogenous nutrition. Peterson et al. (1983) reported that low levels of cadmium (2 ug l^{-1}) were associated with a decrease in larval growth, possibly due to a decreased yolk utilization efficiency. They also reported a decrease in growth after initiation of exogenous feeding, similar to our results. Although little is known about yolk transport of metals, these results suggest that copper and/or zinc may have been transferred in the eggs. Many of the differences reported for MAN larvae are consistent with exposure to low levels of metals, although the larvae were held in clean water.

Upon exposure to copper, the first mortalities for larvae from either site did not occur at the lower concentrations until 9 d post-hatch, the age at which blood is first thought to enter the gill arches (McElman and Balon, 1980). Salmonid sac-fry are also most resistant immediately after hatching (Chapman, 1978). Resistance of larvae increased at 13 d post-hatch and decreased consistently thereafter. All assays of MAN larvae started after the completion of yolk resorption (18 to 20 d) showed decreased resistance relative to larvae tested before this time. Differences in the resistance of fed and unfed larvae were not evident until 29 d.

In this study, 13-d-old larvae exposed to 2200 ug Cu l⁻¹ were twice as resistant as 9-d-old larvae and 14 times more resistant than 29-d-old larvae. Balon's theory of saltatory development (McElman and Balon, 1980) suggests that larvae grow and develop in a series of steps or jumps and that development is not gradual. This is consistent with the fluctuations in resistance which we observed. Variations in the tolerance of young fish to toxicants have been reviewed by Chapman (1978,1985). Holdway et al. (1987) found that the response of suckers to methoxychlor varied over the larval period. In a review of toxicity data, maximum acceptable toxicant concentrations based on early life stage tests were not significantly different from those based on whole life-cycle tests 82% of the time (McKim, 1977). Since variations in larval resistance appear to be rapid, the value of single 96- or 144-h tests conducted during early larval development must be questioned.

White sucker larvae from the metal contaminated site were twice as resistant and showed increased tolerance at 9 and 13 d post-hatch even though the eggs had never been exposed to exogenous metals. Acclimation to external metals has been found for many metals tested and has been in the range of double for copper, cadmium and aluminum, and two to six-fold for zinc (Klaverkamp et al., 1984; Bradley et al., 1985; Chapman, 1985). Acclimation is judged to occur when sublethal exposure results in an increase in tolerance, tolerance being defined as survival at increased toxicant concentrations for an indefinite period of time (Dixon and Sprague, 1981). Short-term increases in resistance may be meaningless, especially if increased resistance times are associated with decreased tolerance (Hickie and Dixon, 1987). In addition to increased resistance to 2200 ug Cu l⁻¹, MAN larvae tested at 9 and 13 d post-hatch showed lower mortality at 600, 900 and 1200 ug Cu l⁻¹, sug-

gesting that these larvae were also exhibiting increased tolerance. Chapman (1985) suggests that acclimation mechanisms may be maximally inducible in embryo/larval fish, and that larval fish may also be inherently more tolerant of some toxicants.

Differences in tolerance and resistance became less apparent as the fish aged and were not detectable at 21 d post-hatch, the age at which the yolk sacs were empty, or 29 d, the last day MAN unfed larvae were alive. If the increased tolerance and resistance of the MAN fish was the result of genetic adaptation, we would expect the increased survival to be evident at all ages tested. Tests on older suckers did not show this to be the case (37 d mortality rates (%) at 8 and 12 h at 2200 ug l⁻¹: MAN (0,80) LOK (0,80), Lake Ontario (20,80) and cross fertilized (0,90)). In fact, the only time periods tested which suggested increases in tolerance and resistance were those conducted during the period of endogenous (yolk) nutrition, or just after (25 d), suggesting the presence of factors in the yolk which confer an advantage to survival. The use of contaminated milt to fertilize control eggs did not result in an increased tolerance or resistance to copper, suggesting that the responsible factors are maternal in origin, and reside in the yolk of eggs collected at contaminated sites. Neither McKim (1977), Rahel (1981) or Klerks and Weis (1987) found cytoplasmic factors or vertical transmission of increased tolerance or resistance in fish.

Examination of the reproductive performance of fish collected at contaminated sites did not detect any differences from controls. Relative to controls, larvae hatched from eggs collected at contaminated sites and fertilized in clean water were smaller, developed faster and exhibited poorer survival, growth and conversion to exogenous food. These changes are all consistent with a hypothesis of food-

limitations at the contaminated sites. During the period of endogenous nutrition, larvae from contaminated eggs showed increased resistance and tolerance to water-borne copper, even though the eggs had never been previously exposed to exogenous elevations of metals. This study provides evidence for a maternal yolk factor associated with increased larval performance to metal exposure. We propose that this factor may be copper and/or zinc which is transferred into the egg in association with yolk-precursors.

Chapter V

EFFECTS OF STREAM INCUBATION ON LARVAE

5.1 Introduction

The 1985 and 1986 egg collections and trials documented a decreased egg size and increased deformity rate among larvae hatching from eggs collected at contaminated sites. The eggs had been fertilized naturally and, based on observations at the time of collection, were fertilized 4 to 7 days before collection. The eggs had been returned to Waterloo for incubation before hatching, and hatching had occurred in clean water. Fuiman and Trojnar (1980) had found that the final size of white sucker eggs was related to the conductivity of the water; as conductivity increased, final egg size decreased. Since Agam Creek flows out of mine tailing ponds, it is probable that the conductivity of the water at MAN spawning sites is higher than at LOK. The 1987 collections involved the water hardening and incubation of MAN eggs in both LOK and MAN water to examine effects on egg size, deformity rate and post-hatch performance of white sucker larvae.

The 1986 testing also suggested that the developmental period from 9 to 20 days post-hatch was a period when the response of the larvae to copper exposure changed several times. The previous testing also suggested that MAN larvae were inherently more Cu-tolerant than LOK larvae during endogenous nutrition, and that the responsible factors were maternal in origin. The phenomenon was not evident in control eggs fertilized with milt from the contaminated site, and tolerance peaked

around the time of the onset of liver functioning (appearance of bile in the gall bladder). A second purpose of the 1987 egg collections was to follow the development of tolerance more precisely and to examine the effects of increasing developmental rate on tolerance and resistance.

This study was initiated to find

1. the effects of stream incubation at the contaminated site on egg size and larval tolerance
2. the effect of temperature on developmental rate and appearance of tolerance.

5.2 Methods

5.2.1 Stream Incubations

Ten females and ten males were collected from Agam Creek (MAN) in late April, 1987. Eggs collected from the females were fertilized in duplicate with a pooled milt sample, and were water hardened with either water from Agam creek (MAN) or from the control site (LOK). After 0.5 hr, the samples were split, and placed in incubation cups in a 60 l cooler containing either MAN or LOK water. Samples were identified with a double-digit code, consisting of two letters, the first representing the site donating the water for water hardening and the second for incubation water (i.e. ML was a sample hardened with MAN water and incubated at the LOK site). An additional sample of control (LL) eggs (LOK water hardened, incubated in LOK water) was transferred by air to the University of Waterloo for incubation.

The incubation cups were placed in cotton mesh bags upstream of the spawning bed, and were anchored in place for 7 d. Eggs were then transported to the Uni-

versity of Waterloo in water from the incubation site. After arrival at the University of Waterloo, the eggs were transferred (d8) to 64 l tubs receiving clean well water at 1 l min^{-1} . This procedure duplicated the 1985 and 1986 collections of eggs from MAN redds. Samples of the eggs were weighed, and the fertilization rate was estimated. After hatch, subsamples were examined for deformation and larval weight.

5.2.2 Cross-fertilizations and field collections

On April 27, six females and five males were collected from Agam Creek (MAN) at 1700 h. Five males from an uncontaminated site (Charon Lake; about 40 km north of Manitouwadge) were collected after 2000 h and fertilizations were performed at 2100 h. The next day the eggs were transferred to the University of Waterloo by air. On May 3, milt from six males at LMN was collected at 1930 h, and crossed with eggs from five females collected at LOK at 2300 h. The LOK eggs were also fertilized with milt collected from six LOK males. The spawning run at LOK was just beginning and more than 100 females were checked to find the five ripe females used. In all cross-fertilizations the gametes were pooled before fertilization (within site only) and water hardening and incubation took place in uncontaminated water.

5.2.3 Incubations and larval rearing

Eggs were incubated following the 1986 procedures with several exceptions. Cross-fertilized eggs, and those from the MAN site were not treated with malachite green for prophylactic fungus control. Eggs which exhibited fungal growth were removed daily. However, extremely low fertilization rates in control eggs (probably associated with the collection of poor milt samples at the beginning of the spawning run) necessitated a single treatment for 1 hr with 1 ppm malachite green.

Approximately 7 d post-fertilization, eggs from the MAN site were divided, and half of the eggs were moved to water 5°C warmer than the rest of the incubation system. Rearing and incubation of these fish took place in 64 l glass aquaria receiving 800 ml min⁻¹ of warmed well water. Larvae were offered food (minced tetramin) beginning 2 d post-swimup.

5.2.4 Bioassays

5.2.4.1 Copper

Larvae were exposed to copper via a modified Mount-Brungs diluter system (McGeachy, unpubl.). Water flow was at a rate of 800 ml min⁻¹, and hardness, alkalinity and pH were measured weekly in all tanks. Mean (se, n) characteristics of the test water were: total hardness 386 (1.4,33) mg l⁻¹ as CaCO₃, calcium hardness 256 (1.0,27) mg l⁻¹ as CaCO₃, magnesium hardness 130 (1.0,27) mg l⁻¹ as CaCO₃ and alkalinity 297 (1.1, 18) mg l⁻¹ as CaCO₃. Temperature of the test tanks were 17 or 12°C. Experimental photoperiod was 16 h light and 8 h dark, with 0.5 h of gradual dawn and dusk incorporated into the light portion.

Bioassays were initiated on MAN larvae every 2 d at the warm temperature and every 2 (MAN) or 3 (LOK) d at the lower temperature. All other groups of larvae were tested at swim-up. For all copper bioassays, nominal concentrations were 0, 450, 900, 1350, 1800 and 2250 ug l⁻¹ copper (prepared from reagent grade CuSO₄·5H₂O). Water concentrations were monitored daily and water samples were acidified to 1% nitric acid and stored in polyethylene bottles for analysis by flame atomic absorption spectrophotometry. Mean assayed concentrations at 12°C (se, n) were 14.4 (3.4,16), 453 (5, 16), 995 (9, 16), 1188 (27,16), 1923 (37, 16) and 2095

(42, 16). Concentrations at 17°C were 22.1 (4.9,16), 461 (8,16), 964 (12, 16), 1314 (20,16), 1846 (29,14) and 2250 (22, 16). The fish were not fed during the 144 hr exposures.

Uptake experiments were initiated on MAN and LOK larvae at swim-up. To monitor uptake, four groups of 30 larvae were placed in the control, 1350 and 2250 $\mu\text{g l}^{-1}$ copper tanks. At 24, 48 and 72 hr (12°C) and at 16, 24, 42 and 48 hr (17°C) larvae were rinsed in clean water and frozen in liquid nitrogen in groups of 10 larvae.

5.2.4.2 Zinc

Several preliminary bioassays using zinc and MAN larvae were unsuccessful in documenting a range of mortality sufficient for analysis. This was due to the marked fluctuations in tolerance during early development: during the first bioassay no mortalities were recorded over the 144 hr period, and during the second bioassay, all larvae were dead after 48 hr. The only successful zinc bioassay involved 17 d old larvae using nominal zinc concentrations of 0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg l^{-1} Zn (made from reagent grade $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$). Measured concentrations (se, n) were .04 (.01,5), .36 (.02,5), 0.48 (.05,5), 1.10 (.09,5), 2.20 (.11,5) and 3.92 (.09,5) mg l^{-1} Zn.

5.2.5 Metal analysis

Larval samples were dried for 60 h at 55°C, weighed and transferred to 125 ml flasks. Samples were digested in 3 ml 1:8 (conc. H_2SO_4 : conc. HNO_3) in a perchloric fume-hood until clear. Samples were refluxed using small glass funnels at 200-220°C for approximately 2 h, and several drops of 30% H_2O_2 were added at 5 min intervals. Samples were diluted to 50 ml with distilled water, and tissue cop-

per concentrations were determined using a Perkin-Elmer 4000 Atomic Absorption Spectrophotometer at 324.8 nm. Measurements using these techniques gave a value which was 97% of the value reported for NBS Bovine Liver Standard (Lot 1577a) (Miller et al., 1988).

5.3 Results

The fertilization rate of MAN eggs was (se, n) 76.4% (4.7,10). The fertilization rate of the pool of MAN eggs used for cross-fertilization was 85%, while Charon Lake males fertilized eggs at a rate of 98%. The fertilization rate of LOK eggs was very low (<10%), while the fertilization of LOK eggs with milt from LMN males resulted in >80% success. LOK males, similar to 1986 collections, yielded less than 1 ml milt per collection, suggesting that the release of milt early in the spawning run at LOK is not sufficient to suggest readiness of the males for spawning.

There was no effect of water hardening ($p=0.55$) or incubation site ($p=0.39$) on the fertilization rate of the MAN eggs (Table 21). Incubation at the MAN site was associated with a decreased egg size ($p=0.02$), and the effect of water hardening was marginal ($p=0.054$). There was no effect on subsequent larval size between the field sites (Table 21), although there was an interaction between the water hardening and incubation sites ($p=0.02$). A breakdown of the analysis showed that there was no effect of water hardening site on larval size ($p=0.10$), but that larvae incubated at LOK were significantly smaller than those from the laboratory incubation ($p=0.015$). There were no differences in weight of LOK and MAN larvae.

There was no difference in the deformity rates of MAN eggs incubated at LOK or in the laboratory ($p>0.25$), but both exhibited significantly fewer deformities than

Table 21. Effects of water hardening at the contaminated site (MAN) on the fertilization rate, egg size and larval weight of white sucker eggs collected at MAN. Values are given as the mean \pm s.e. (n). Values sharing an alphabetical superscript are not significantly different.

Water Hardening Site	Incubation Site	Fertilization Rate (%)	Egg Weight (mg)	Larval Weight (mg)
LOK	Laboratory	76.4 \pm 4.7 (10) ^A	18.2 \pm 0.4 (10) ^A	7.49 \pm 0.17 (40) ^A
LOK	LOK	67.1 \pm 3.6 (10) ^A	18.5 \pm 0.4 (10) ^A	7.16 \pm 0.17 (40) ^B
MAN	LOK	73.7 \pm 4.6 (10) ^A	17.9 \pm 0.4 (10) ^A	6.73 \pm 0.11 (40) ^B
LOK	MAN	73.4 \pm 3.4 (10) ^A	17.5 \pm 0.4 (10) ^B	7.00 \pm 0.15 (40) ^{A B}
MAN	MAN	71.3 \pm 5.1 (10) ^A	16.8 \pm 0.4 (10) ^B	7.24 \pm 0.13 (40) ^{A B}

larvae from the MAN site ($p < 0.005$) (Figure 13). The results were identical to comparisons of eggs collected from LOK redds in 1986 and laboratory incubations with the MAN redd collections from 1985 (significant increase, $p < 0.0001$). The MAN incubation water had a higher copper and zinc concentration than the control site, and a higher total hardness (Table 22).

Laboratory exposure of the larvae to copper suggested that eggs water hardened at the contaminated site exhibited a decreased tolerance to copper, regardless of incubation site (Table 23). However, eggs incubated at the contaminated site exhibited a decreased resistance, regardless of water hardening site (Table 23). Incubation and water hardening of sucker eggs at the contaminated site was associated with an increased egg metal burden (Table 24). The dry weight of LOK larvae (.99 mg, .08, s.e., 11, n) was significantly higher than MAN larvae (.69, .06, 11) ($p = 0.02$).

An increase in the water temperature accelerated the developmental rate of MAN suckers, and developmental times were similar to the 1986 trials (Table 17, 25). Increased temperature accelerated development, but there was no apparent shift in the peak resistance time (Figure 14). After the swim-up of larvae at the warmer temperature, both the resistance and tolerance of the suckers was decreased, relative to the larvae at the cooler temperatures (Table 25).

During the uptake experiments LOK larvae consistently contained lower copper body burdens in control water (20.3 mg kg⁻¹, 11.2, s.e., 4, n), and at concentrations of 1350 (42.6, 16.2, 4) and 2250 ug l⁻¹ (85.6, 46.1, 3) compared to MAN larvae (37.5, 8.3, 3; 71.2, 2; 145.0, 43.6, 3).

Figure 13:

Deformity rates of sucker eggs collected at the spawning sites. During 1986 collections, eggs from LOK and MAN were fertilized and hardened in water from the control site. The MAN redd collections (from Agam Cr.) were eggs collected from the spawning sites which had been naturally fertilized 4 to 7 days before collection. The 1987 collections correspond to MAN eggs fertilized in LOK water, MAN eggs fertilized in MAN water but incubated at LOK, and MAN eggs fertilized in MAN water and incubated at MAN.

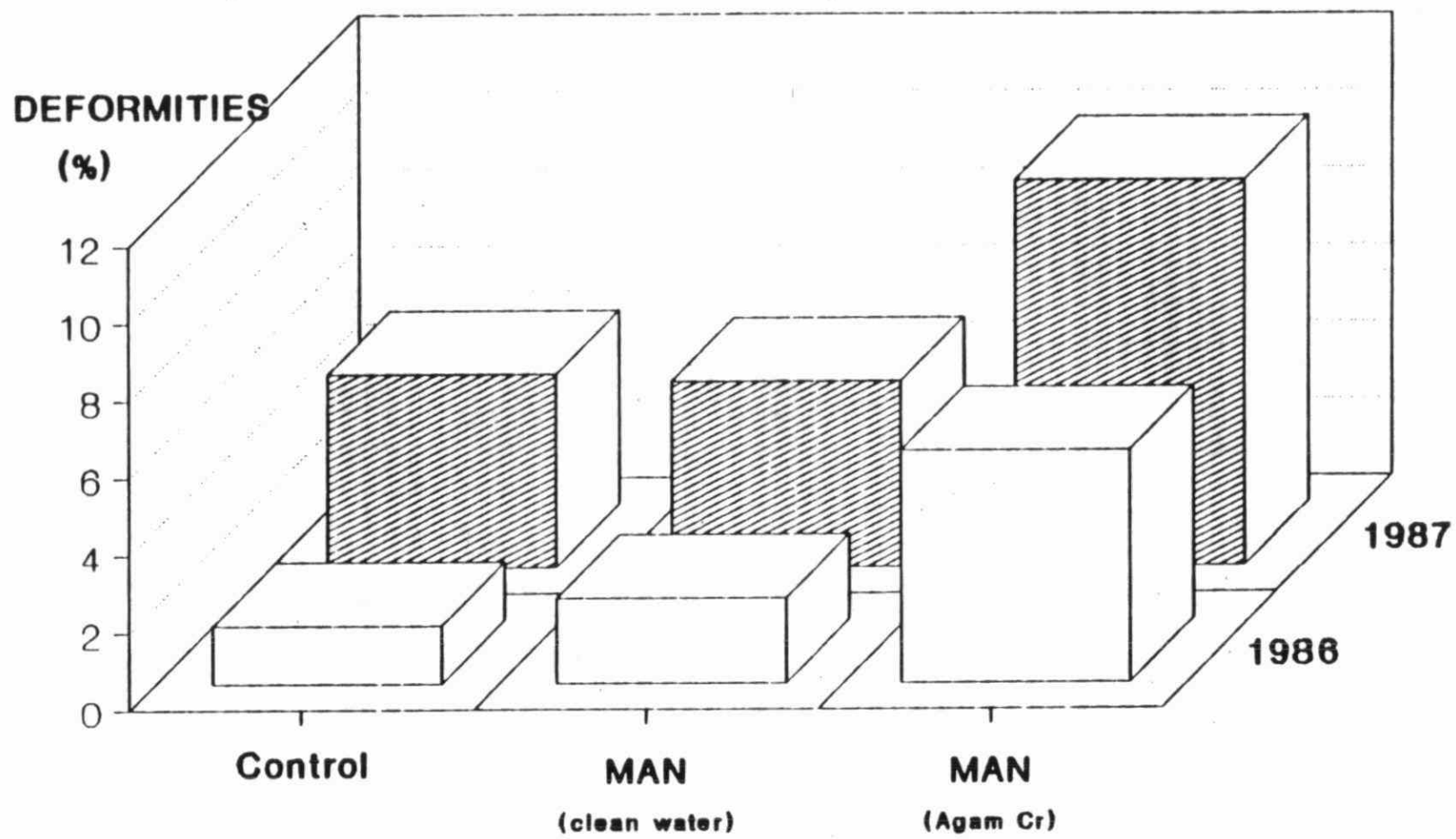


Table 22. Metal levels ($\mu\text{g l}^{-1}$, total unfiltered metals) and total water hardness (mg l^{-1} as CaCO_3) of water samples collected during the field incubation experiments. Values are given as the mean \pm s.e. (n).

Site	Copper	Zinc	Hardness
LOK	4.0 ± 2.7 (3)	2.7 ± 1.4 (3)	95.8 ± 0.6 (5)
MAN	13.0 ± 5.3 (6)	400.3 ± 17.8 (6)	296.0 ± 0.5 (5)

Table 23. Effect of water hardening or incubation in contaminated water on the response of white sucker larvae to copper exposure. Values in brackets represent the 95% fiducial limits. All eggs are from the identical batch of MAN eggs which were split before water hardening and incubation. Values sharing a superscript are not significantly different.

Water Harden Site	Incubation Site	Trial 1 (12 d old)			Trial 2 (16 d old) (Swim-up)		
		LT50 (2250 ppb)	96 hr LC50	144 hr LC50	LT50 (2250 ppb)	96 hr LC50	144 hr LC50
LOK	LOK	75.9 ^a (71.0-81.2)	2074 ^a (1857-2315)	1210 ^b (1125-1302)	95.1 ^A (79.1-114.3)	1899 ^A (1791-2013)	1264 ^A (1124-1421)
MAN	LOK	69.8 ^a (58.6-82.9)	1393 ^c (1181-1641)	1046 ^c (973-1123)	72.7 ^B (67.9-77.9)	1867 ^A (1574-2214)	1179 ^A (1088-1276)
LOK	MAN	43.5 ^b (40.1-47.7)	1627 ^b (1441-1836)	1371 ^a (1279-1468)	52.0 ^C (29.8-90.8)	1924 ^A (1574-2352)	1239 ^A (1098-1399)
MAN	MAN	38.1 ^b (29.9-48.4)	1344 ^c (1205-1498)	1091 ^c (1011-1177)	58.3 ^C (55.4-61.3)	1319 ^B (1196-1455)	1029 ^B (971-1091)

Table 24. Gonadal metal burdens, expressed as mean \pm s.e. (n), mg kg⁻¹ on a dry weight basis.

	Season	MAN		LOK	
		Copper	Zinc	Copper	Zinc
<hr/>					
<u>Testes</u>	POST	15.8±3.7 (4)	89.3±13.2 (4)	8.5±1.5 (2)	136.5±25.5 (2)
<u>Ovaries</u>	PRES	6.4±0.5 (5)	113.6±6.2 (5)	7.0±1.0 (2)	84.5±3.5 (2)
	POST	18.8±2.7 (5)	290.0±78.3 (5)	21.0±6.0 (2)	316.5±11.5 (2)
<u>Eggs</u>	SPA ^W 1	10.7±0.8 (6)	83.3±11.9 (6)	8.0±0.5 (5)	69.4±2.6 (5)
	SPA ^W 2	50.7±1.9 (3)	158.4±3.0 (7)	18.3±5.1 (4)	108.4±4.1 (7)
<u>Larvae</u>		49.5±15.5 (7)	511.1±96.0 (7)	28.0±9.7 (5)	163.2±29.4 (5)

¹eggs only, collected before fertilization or water hardening

²eggs only, collected from stream bed after natural fertilization and water hardening, controls from Lake Ontario

Figure 14: Effects of age and temperature on resistance to copper. The clear boxes represent larvae reared at 12°C and the shaded boxes represent larvae reared at 17°C. Values represent resistance time (LT50) at 2250 $\mu\text{g l}^{-1}$ Cu.

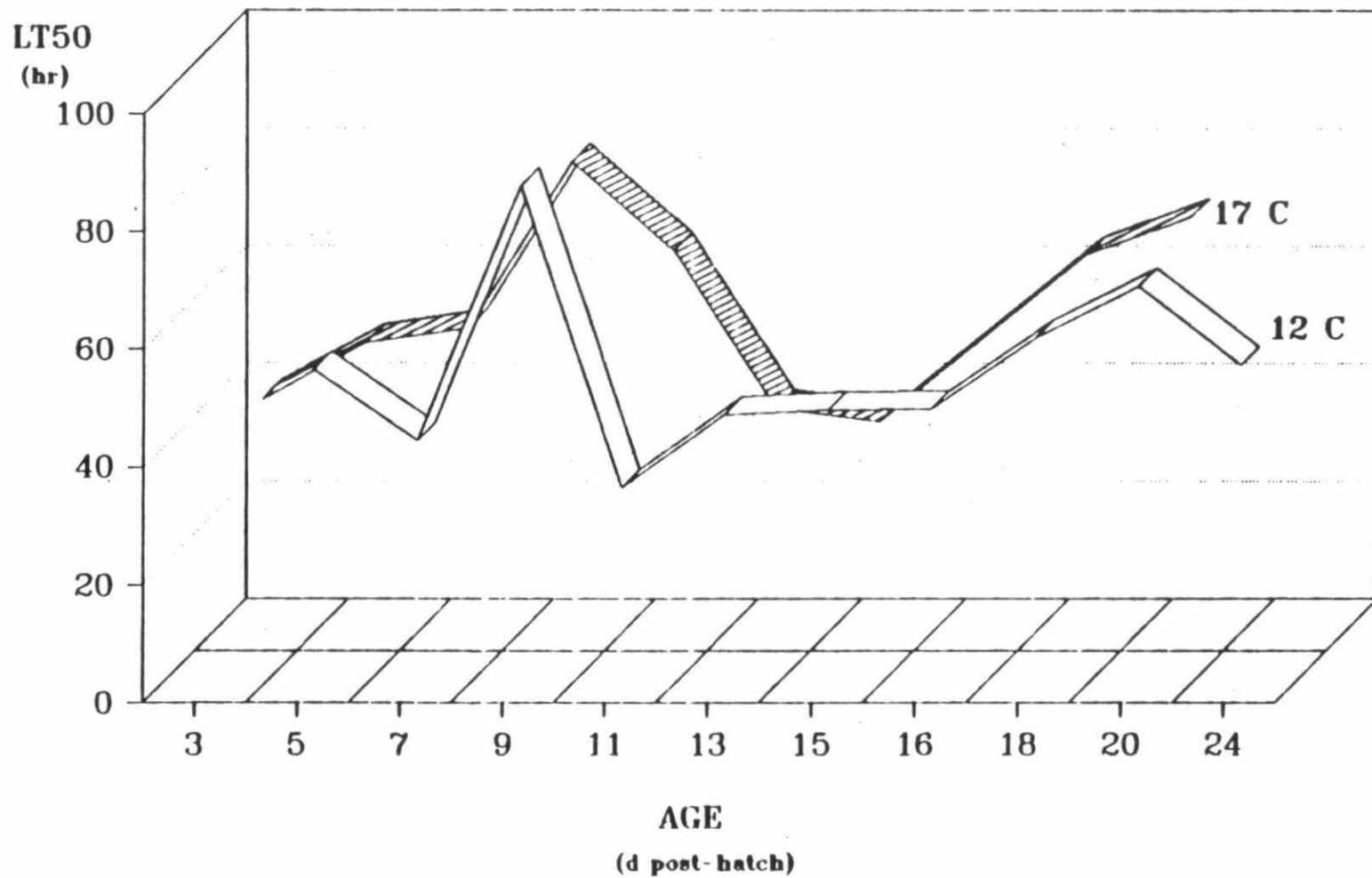


Table 25. Effect of age and developmental stage on the resistance and tolerance of white sucker larvae to copper. Values in brackets represent the 95% fiducial limits. Within columns, values without an alphabetical superscript in common are significantly different. Values marked with an asterisk are significantly different from the comparative value at the higher temperature.

Incubation Temperature		10-12°C			16-17°C			
Age ¹	DTU ²	Stage	LT50 (2250 ppb)	LC50 (% hr)	DTU	Stage	LT50 (2250 ppb)	LC50 (% hr)
3	30				48	First Swimming	39.9 ^a (37.1-42.9)	>1800 ³
						Consistent Swimming		
5	50	First swimming	53.7 ^c (50.0-57.7)	1467 ^{ab*} (1277-1686)	80	Bile production	49.4 ^c (45.5-53.6)	1988 ^a (1889-2092)
7	72	Consistent Swimming	41.6 ^{d*} (36.9-46.8)	1412 ^{ab} (1311-1520)	112	Fins moving Swimming at surface	52.0 ^c (49.1-55.0)	1497 ^b (1266-1769)
9	94	Bile production	84.9 ^a (78.2-92.1)	1723 ^a (1359-2184)	144	Swim-up	80.3 ^a (77.5-83.2)	1854 ^a (1603-2145)
11	117	Fins moving	33.6 ^{a*} (29.3-38.7)	1400 ³	177		65.2 ^b (62.8-67.6)	724 ^{cd} (579-907)
13	141	Swimming at surface	46.1 ^d (42.9-49.5)	1168 ^{cd*} (1081-1267)	211		38.3 ^d (30.7-47.7)	748 ^c (637-880)
15	165	Swim-up	46.9 ^{d*} (43.4-50.7)	1249 ^{cd*} (1135-1375)	245	First feeding	36.0 ^d (32.4-40.0)	452 ^d (298-687)
16	177		47.0 ^d (42.0-53.0)	>1340 ³	262		.4	.4
18	201		58.9 ^c (55.3-62.7)	1406 ^{abc} (1269-1556)	296		64.5 ^b (58.1-71.5)	.4
20	225		67.9 ^b (63.6-72.5)	1119 ^d (939-1333)	330		70.9 ^b (64.7-77.6)	.4
24	273	First feeding	54.5 ^c (50.5-58.8)	1310 ^{bcd} (1197-1433)				

¹days post-hatch

²daily temperature units

³estimated

⁴not enough fish left

Early in development (5d post-hatch), LOK larvae exhibited a resistance time (LT50) at the highest concentration (2250 ug l⁻¹) of 26.9 hr (fiducial limits 22.7-31.9) and LOKxLMN larvae 10.8 hr (8.5-13.7) relative to the MAN time of 53.7 hr (50.0-57.7). Both groups of larvae also exhibited decreased resistance and/or tolerance times at later ages of development (Table 26). The cross-fertilization of MAN eggs with Charon Lake males yielded larvae which showed no apparent differences from the MANxMAN larvae (Table 26). The very low fertilization rate of LOK larvae prevented a detailed comparison of the larval groups, although such testing was accomplished in 1986.

Table 26. Effects of collection source on resistance and tolerance of sucker larvae to copper. Values in brackets represent the 95% fiducial limits. Values within a time period sharing an alphabetical superscript are not significantly different.

Age (d)	Source		LT50 (2250 ppb)	96 hr LC50	144 hr LC50
	Males	Females (age)			
8-10 d	MAN	MAN (9d)	84.9 ^a (78.2-92.1)	1723 ^a (1359-2184)	977 ^a (772-1236)
	LMN	LOK (10d)	51.0 ^b (48.0-54.1)	---	---
	LOK	LOK (8d)	41.4 ^c (37.9-45.1)	1066 ^b (931-1221)	780 ^a (661-921)
	LOK	LOK (10d)	48.4 ^b (44.2-53.0)	1027 ^b (907-1164)	757 ^a (641-894)
13 d	MAN	MAN	46.1 ^a (42.9-49.5)	1168 ^a (1081-1262)	880 ^a (741-1046)
	Charon	MAN	40.1 ^a (35.2-45.6)	1049 ^b (1004-1095)	900 ¹
	LOK	LOK	31.9 ^b (28.2-36.2)	932 ^b (813-1068)	752 ^a (642-829)
16-17 d	MAN	MAN (16d)	47.0 ^a (42.0-53.0)	>1340 ¹	951 ^a (867-1119)
	Charon	MAN (17d)	45.5 ^a (43.2-48.0)	1479 (1316-1529)	968 ^a (876-1070)

¹estimated

5.4 Discussion

Incubation of eggs at the MAN site was associated with a decrease in egg size, but there was no effect on larval size or fertilization rate. The changes were consistent with differences in water hardness, and are consistent with previous findings that conductivity was inversely related to the final size of white sucker eggs (Fuiman and Trojnar, 1980).

Visual observations of the eggs during water hardening noted that no changes in egg size occurred after 15 min. Despite this observation, 0.5 hr of water hardening was not sufficient to prevent entrance of water. Incubation of eggs at the MAN site was associated with a decreased size and copper tolerance regardless of water hardening site. Eggs which were incubated and water hardened at MAN, but hatched in clean water, exhibited an increased deformity rate and a decreased tolerance relative to eggs incubated at either LOK or in the laboratory. This suggests that some metals entered the egg during water hardening.

Water exchange obviously continues to occur for a period of time after the initial water influx. Egg metal levels were higher in eggs after water hardening than before, although the location of the metals may be in the peri-vitelline space. The highest metal burdens were found in larvae after hatching, which suggests that some of the metals entering the egg during water hardening do become associated with the larvae.

The major type of deformity at MAN and LOK was a "c"-shaped deformity. The incidence ranged from 87 to 100% of deformity types, which was much higher than the prevalence in larvae hatched from Lake Ontario eggs. The reasons for this are not known, but it may be related to the different contaminant burdens present in the Great Lakes.

Developmental effects on tolerance followed the same pattern as in 1986. Although an increase in incubation temperature shifted development, the pattern of changes in resistance remained the same. This defies logical explanation, unless the changes in resistance are associated with some factor other than developmental changes within the larvae. The peaks in resistance were slightly closer together at the higher temperature (Figure 14), but the difference was not significant. The absence of a shift may have been associated with the delay in transfer to the higher temperature until 7 d after fertilization.

As was evident in the 1986 collections, the transfer of tolerance was associated with the MAN eggs and not the milt. The effect was not evident in control eggs crossed with contaminated milt, but seemed to be present in contaminated eggs crossed with control milt. Incubation at the contaminated site was associated with a highly significant decrease in tolerance, although the MAN larvae still exhibited an increased tolerance over LOK larvae.

Although the benefits of acclimation to adult fish have been questioned (Sprague et al., 1984), acclimation of larval fish may be much more important. The loss of natural tolerance in the fish from the contaminated sites coincides with the time of swim-up and downstream movement. The transfer of tolerance via the yolk allows increased survival when the ambient levels may not be consistently high enough to illicit normal acclimatory responses (0.5 LC50, Sprague et al., 1984). The identity of the factor associated with the altered response is unknown. Changes in tolerance have been associated with fluctuations in levels of metallothioneins (MT) (Buckley et al., 1982; McCarter and Roch, 1984; Bradley et al., 1985), but it is now thought that the rate of MT synthesis is more important than the actual concentration

(McCarter and Roch, 1984). This suggests that the identity of the factor is not MT, since the transfer of maternal MT would not be as advantageous to the offspring as the synthesis. Many contaminants can be transferred to the offspring through the egg (Niimi, 1983), and the factor may be the metals themselves. Cross-acclimation between metals is possible, and Pierson (1981) found that pregnant guppies (Poecilia reticulata) actively transferred zinc to embryos, and interpreted acclimation from the absence of a decreased growth rate. Levels of copper and zinc are significantly higher in eggs collected from MAN redds than control eggs but the location of the metals has yet to be identified. The peak tolerance at 9 and 13 d post-hatch is associated with the onset of liver function, and it is possible that the mobilization of metals out of the yolk may stimulate the larval liver to synthesize MT. It is unclear whether the loss of resistance with time is the result of a loss of the yolk factors or a dilution of the responsible factors with growth of the larvae.

MAN incubation was associated with a decrease in egg size and larval tolerance, but did not affect larval size or fertilization rate. The effects on MAN larvae, and the changes in tolerance were consistent with previous findings. Shifting temperature shifted development, but did not alter the resistance profile.

Chapter VI

COPPER AND ZINC DISTRIBUTION IN SUCKER TISSUES

6.1 Introduction

The role of metals, both as micronutrients in fish nutrition and as potential aquatic toxicants, is well recognized and has been widely investigated (Leland and Kuwabara, 1985). The majority of work has centered on the nutrient requirements and/or toxicity of single metals, with little consideration of interactions among metals. Copper and zinc are often both elevated in environments subjected to contaminant input, particularly as a result of mining activity. Mammalian studies have reported that antagonism occurs between Cu and Zn during dietary uptake (Mertz, 1986), such that increased dietary levels of zinc can reduce hepatic copper concentrations (Bremner et al., 1976). There is also some limited evidence that Cu - Zn antagonism occurs during uptake of the two metals from the gastrointestinal tract in fish (Shears and Fletcher, 1979; Knox et al., 1984). There is little information available on Cu - Zn interactions in situations where fish are exposed to elevated levels of the two metals in both diet and water. Neither is there information on metal levels in tissues of wild fish from environments contaminated with both Cu and Zn.

The present study examined the Manitouwadge system of lakes in northern Ontario, a system which has been subjected to mining-associated contamination by

copper and zinc since 1957 (German, 1971). In previous work on this system we have shown significant impacts on the white sucker (*Catostomus commersoni*) populations in the metal-contaminated lakes. White sucker have received some attention as a sentinel species for environmental contamination (McFarlane and Franzin, 1978, 1980; Schmitt et al., 1984), since they are relatively long-lived, intermediate in the food web and, as benthic feeders, in contact with sediments (Ney and Van Hassel, 1983). In all the lakes which we studied, the sucker reached maturity between 4 and 6 years of age, and until 6 years of age there were no differences in length or weight of fish collected from control and contaminated lakes. After this age, fish from contaminated sites were significantly smaller and shorter than those from control sites. In addition, female sucker from contaminated lakes failed to exhibit significant increases in either length or weight after the age of reproductive maturity. The fish from contaminated lakes also exhibited decreases in egg size and fecundity, no significant increases in fecundity with age, and an increased incidence of spawning failure (Munkittrick and Dixon, 1988a). Larvae hatched from eggs collected at contaminated sites were smaller, developed at a slightly increased rate and exhibited poorer growth and survival than larvae from control sites. They also showed increased tolerance of copper, relative to controls, during the period of endogenous nutrition (Munkittrick and Dixon, 1988 b).

During this study we collected white sucker from control and contaminated lakes within the system to determine tissue levels of Cu and Zn. This was undertaken to characterize the tissue metal profiles of wild sucker simultaneously exposed to Cu and Zn, and to look for evidence of Cu - Zn antagonism. Since sucker are benthic feeders, special attention was paid to metal levels in sediments and gastrointestinal tract contents.

6.2 Methods

6.2.1 Fish Collections

The control lake for this study was Loken Lake (LOK; mean (S.D.) pH, 7.2 (0.3); total hardness, 85 (23) mg L⁻¹ as CaCO₃) while Manitouwadge Lake (MAN; pH, 6.9 (0.4); total hardness, 112 (41) mg L⁻¹ as CaCO₃) was chosen as the contaminated lake. White sucker were collected with a 8.8 cm monofilament gill net during September of 1986. Fish were anesthetized in a solution of tricaine methane-sulphonate (MS 222), and the weight (nearest 5 g) and standard length (nearest 0.5 cm) recorded. The weights of the fish ranged from 490 to 1420 g with a mean (S.E.) of 786 (141) g. There was no significant differences ($p = 0.43$) in the weights of the fish between sites.

The left pectoral spine was removed at the base, dried and sectioned with a 7/0 jeweller's saw. Annuli were counted to determine the age of each sucker. Samples of liver, muscle, kidney, spleen, gall bladder/bile, visceral lipid, gill and ovary were taken. The gastrointestinal tract was subdivided such that 4 samples of digesta were obtained: stomach, the anterior 5 cm of the intestine, the 5 cm portion of the intestine just posterior to the loop and the remaining posterior portion of the intestine (feces). All samples were immediately frozen in liquid nitrogen and stored at 20°C pending metal analysis.

Sediment and water samples for determination of Cu and Zn content were taken from randomly selected sites in each lake. Water samples (unfiltered) were collected at a depth of 0.5 m, acidified to 1% with nitric acid and analyzed by flame atomic absorption spectrophotometry (FAAS). Sediment samples were collected with a 5.4 cm diameter core sampler and immediately frozen in liquid nitrogen. Total non-

residual metal levels were measured in the sediments. Each sample was thawed and passed through an 80-mesh sieve. A 1.0 g sample of the material net retained by the sieve was placed in 100 mL of 0.5 N HCl. The sample was shaken for 16 h, filtered through a 0.45 μ m cellulose acetate filter and the leachate analyzed for Cu and Zn content by FAAS.

6.2.2 Tissue analysis

The Cu content of tissues were determined by either neutron activation analysis (NAA) or FAAS; Zn levels were determined exclusively by FAAS. NAA used the facilities at McMaster University, Hamilton, Ontario. Prior to analysis, tissues were oven dried for 60 h at 80°C and individually packaged in sealed 2 cm diameter polyethylene vials. The samples were irradiated for 30 sec at a flux of 1.3 MW (ca. $4.5 \times 10^{12} \text{ cm}^{-2} \text{ sec}^{-1}$). Copper was determined as ^{66}Cu by analyzing the gamma spectra at 1039 keV in a Canberra spectrophotometer. Copper concentrations were derived using an in-house computer program standardized with reference samples from the National Bureau of Standards (NBS) and the National Research Council.

Prior to analysis by FAAS, dried tissue samples were digested in 5 mL of mixed H_2SO_4 and HNO_3 (1:8, 36 N H_2SO_4 : 15.9 N HNO_3 , V:V). The samples were refluxed for 2 h at 210°C and cleared addition of 0.5 mL of 30% H_2O_2 . These samples were diluted to 50 mL with double-distilled water and Cu and/or Zn concentrations determined using a Perkin-Elmer 4000 AAS. Analysis of NBS bovine liver standards by our techniques yielded recoveries of 99 and 97 % of nominal for Cu and Zn respectively. Since there was no significant differences in Cu concentrations between tissues analyzed by both FAAS and NAA ($p=0.90$), the data were pooled.

Log transformed data were subjected to analysis of variance using the SAS statistical analysis package at the University of Waterloo. Digesta metal levels were compared using a repeated measures program (Zar, 1984), and regressions of metal burden on age and body size were performed on log transformed values.

6.3 Results

The mean concentrations of Cu and Zn in both the water and sediments of the contaminated lake (MAN) were significantly elevated relative to the concentrations apparent at the control lake (LOK) (Table 27).

White sucker from MAN had significantly higher levels of both Cu and Zn in liver, kidney, gill and the digesta from all segments of the gastrointestinal tract, relative to sucker from LOK (Table 28). There were no significant differences between lakes for the levels of either metal in visceral lipid or gall bladder/bile of sucker. Although lake had no impact on ovarian Cu concentration, the level of Zn was elevated in the ovaries of fish from MAN. While there was no effect of lake on the muscle Cu content of sucker, the levels of Zn in muscle of fish from the control (LOK) lake were, most interestingly, significantly elevated relative to those from the contaminated (MAN) lake. Along the same line, control fish had significantly higher Cu concentrations in spleen than fish from MAN. Site had no effect on the concentrations of zinc in spleen.

There was no effect of weight ($p=0.73$), length ($p=0.94$) or age ($p=0.39$) on liver Cu concentration at MAN. Similar trends were evident at LOK. All three factors were consistently positively correlated for males and negatively correlated for females, although the relationships were not significant. There were, in addition, no

Table 27: Mean (S.E., n) concentrations of Cu and Zn in water and sediments from the control (LOK) and contaminated (MAN) lakes.

Lake	Metal	Water concentration ($\mu\text{g L}^{-1}$)	Sediment concentration ($\mu\text{g g}^{-1}$)
MAN	Cu	15 (4, 35)	102 (24, 18)
	Zn	253 (62, 35)	1149 (329, 18)
LOK	Cu	<2 (-, 11)	11 (3, 18)
	Zn	26 (22, 11)	43 (7, 18)

Table 28: Tissue levels $\mu\text{g g}^{-1}$ dry weight) of Cu and Zn, as well as Cu:Zn ratios for white sucker collected from contaminated (MAN) and control (LOK) lakes. Tissue levels are given as means (S.E., n). The p values represent the probability that the means between sites were the same.

Tissue	Cu ($\mu\text{g g}^{-1}$)			Zn ($\mu\text{g g}^{-1}$)			Cu : Zn	
	MAN	LOK	P	MAN	LOK	P	MAN	LOK
liver	83 (6, 34)	50 (7, 22)	0.001	210 (14, 11)	112 (9, 14)	0.002	1:2.5	1:2.2
gall bladder	40 (16, 5)	17 (3, 9)	0.075	70 (16, 4)	47 (17, 6)	0.248	1:1.8	1:2.8
kidney	26 (4, 39)	14 (2, 20)	0.009	192 (17, 8)	97 (7, 7)	0.001	1:7.4	1:6.9
gill	15 (2, 10)	6 (1, 6)	0.001	138 (14, 10)	66 (18, 6)	0.007	1:9.2	1:11.0
muscle	6 (1, 13)	7 (1, 13)	0.212	20 (3, 6)	25 (2, 6)	0.038	1:3.3	1:3.6
spleen	7 (1, 7)	10 (1, 5)	0.010	78 (10, 7)	72 (9, 5)	0.687	1:11.1	1:7.2
visceral lipid	5 (3, 4)	10 (4, 4)	0.399	21 (4, 4)	37 (10, 4)	0.189	1:4.2	1:3.7
ovary	9 (1, 11)	8 (1, 7)	0.350	97 (8, 11)	74 (3, 7)	0.056	1:10.8	1:9.3
stomach (contents)	155 (53, 6)	9 (7, 4)	0.020	886 (175, 6)	21 (12, 4)	0.010	1:5.7	1:2.3
anterior intestine (content)	228 (27, 4)	30 (7, 4)	0.001	2130 (461, 4)	112 (22, 4)	0.001	1:9.3	1:3.7
posterior intestine (contents)	244 (40, 4)	39 (23, 4)	0.001	1708 (308, 4)	80 (30, 4)	0.001	1:7.0	1:2.1
feces	231 (29, 6)	49 (21, 4)	0.001	1821 (263, 6)	105 (53, 4)	0.001	1:7.9	1:2.2

effects of weight ($p = 0.48$), length ($p = 0.13$) or age ($p = 0.48$) on the liver concentration of Zn at MAN, with similar trends at LOK.

Digesta collected from the stomach, anterior intestine, posterior intestine and lower intestine (feces) of MAN sucker were examined for partial correlation of metal levels and position in the digestive tract. There was a marginal correlation between Cu levels and position in the digestive tract with significance ($p=0.001$) only occurring between the posterior intestine and feces (Table 29). The stomach Zn concentration negatively correlated with all other regions of the gut. Fecal Zn concentration was correlated with posterior intestine ($p=0.0002$). For both Cu and Zn, the correlations were highest for posterior intestine and feces ($r=0.97$ for both) (Figures 15,16).

Table 29: Correlation coefficients (r) and significances (p) for correlations of copper (A) and zinc (B) concentrations in the stomach (STO), anterior intestine (ANT), posterior intestine (POS) and feces (FEC) of white sucker (N=4) from the contaminated lake.

A. Copper		ANT	POS	FEC
STO	r	0.186	0.428	0.637
	p	0.690	0.340	0.124
ANT	r		0.653	0.624
	p		0.110	0.134
POS	r			0.966
	p			0.001

B. Zinc		ANT	POS	FEC
STO	r	-0.859	-0.804	-0.875
	p	0.013	0.020	0.001
ANT	r		0.704	0.690
	p		0.070	0.080
POS	r			0.973
	0			0.002

Figure 15: Copper distribution in liver, gall bladder and digestive tract.. Numbers represent means and sd (n).

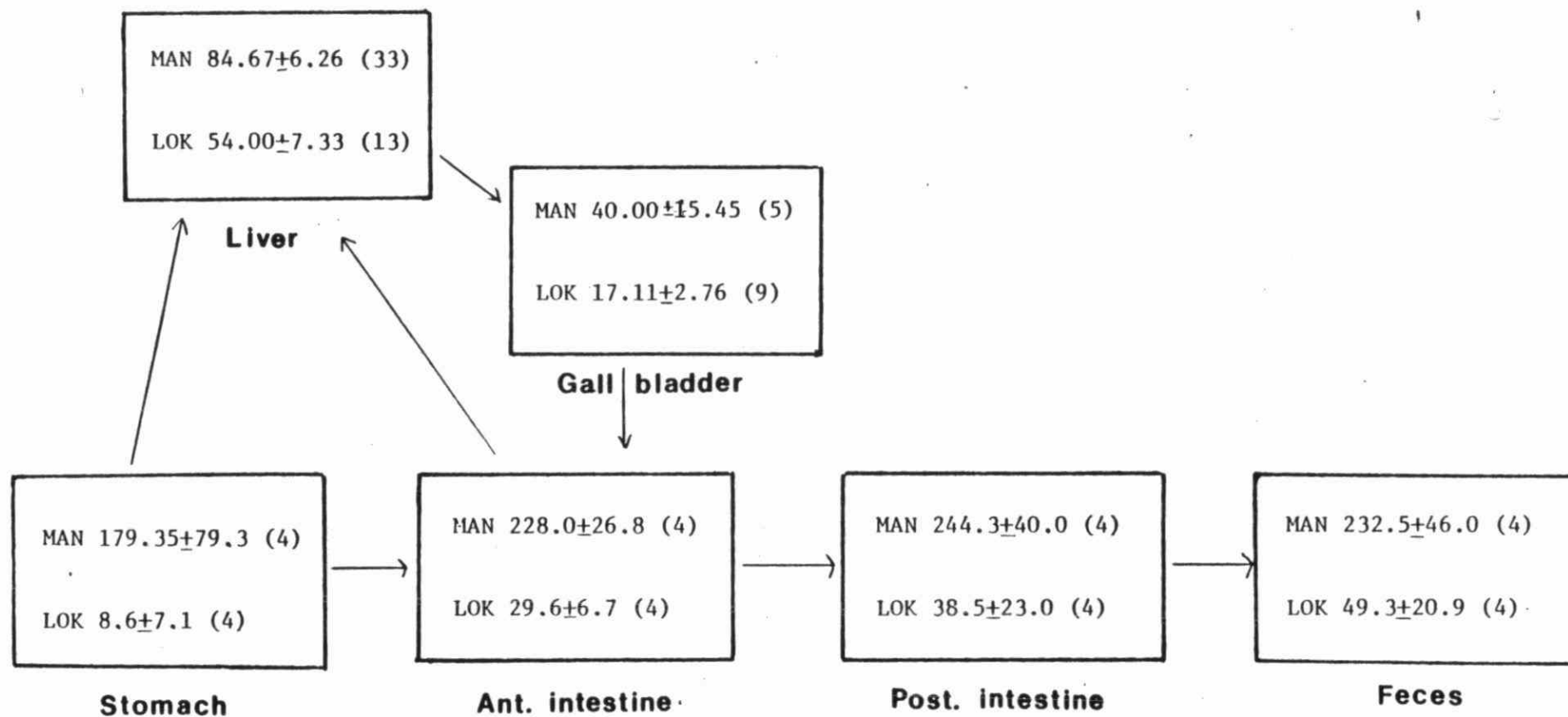
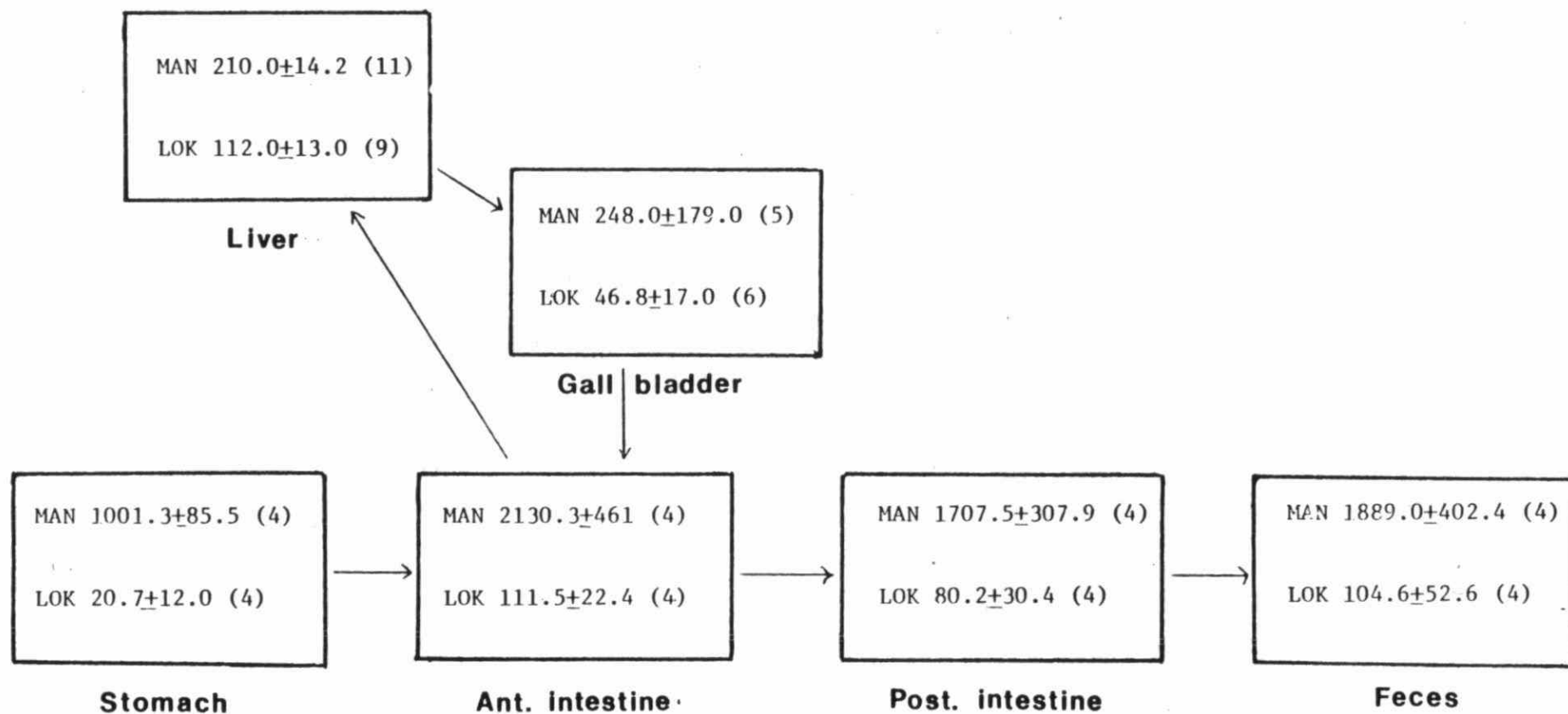


Figure 16:

Zinc distribution in liver, gall bladder and digestive tract.. Numbers represent means and sd (n).



6.4 Discussion

The elevated levels of Cu and Zn in both water and sediment at the contaminated (MAN) lake, relative to the control (LOK) lake, were to some extent reflected in the tissue metal levels of white sucker from the two sites. The fish from MAN showed significantly increased levels of both Cu and Zn in liver, kidney and gill tissue. Similar increases in levels of Cu and Zn in metabolic tissues have been reported for other species of fish taken from contaminated environments (Wilson, 1980; Roch and McCarter, 1984).

Despite the elevated metal levels in metabolic tissues, the levels of Cu and Zn exposure at the contaminated lake would appear to fall within the range of homeostasis for the sucker. Levels of Cu and Zn in visceral lipid, and of Cu in ovary, were not elevated in fish from the contaminated site. The levels of Cu in spleen and Zn in muscle were, in fact, significantly higher in control sucker than they were in sucker from the contaminated site. This is consistent with reports that, within the range of tolerance for nutritionally essential metals such as Mn, Fe, Zn and Cu, normal homeostatic mechanisms allow isolation of the muscular compartment from environmental elevations (Murphy et al., 1978; Wilson, 1980).

Further evidence for the maintenance of homeostasis lies with the Cu: Zn ratios in the tissues studied. Despite the fact that the Cu:Zn ratio was greater in the diet (stomach contents) at MAN (1:5.7) than at LOK (1:2.3), and with the exception of gall bladder/bile, the Cu:Zn ratios were very similar between sites for all of the tissues examined (Table 28). The increased proportion of Cu relative to Zn in gall bladder/bile at MAN may reflect the biliary-fecal excretion of Cu (Evans, 1973).

There were no significant relationships between age or body size and the concentration of Cu or Zn in liver. The relationship of metal concentration with age and body size of fish can vary with metal (Cross et al., 1973, Leland et al., 1978), species of fish (German, 1971, Cross et al., 1973, McFarlane and Franzin, 1980), sex, size and season (Shears and Fletcher, 1983) and age of fish (Ney and Van Hassel, 1983). While the range of weights of the fish in this study (490 to 1420 g) may have been too narrow to detect a relationship, the absence of a relationship further supports the concept that the environmental metal levels were within the regulatory capabilities of the fish.

The high levels of Cu and Zn in both MAN sediments and the stomach contents of MAN sucker strongly suggest diet as a metal entry-point for these fish. Although considerable controversy exists with respect to the relative contributions of diet and water to metal uptake in fish (Baudin, 1987), dietary input can represent a significant source (Patrick and Loutit, 1978; Dallinger and Kautzky, 1985). In plaice (*Pleuronectes platessa*), diet can represent the major source of Zn up to water concentrations of 0.6 mg L^{-1} (Milner, 1983). Furthermore, the metal levels in plankton and invertebrates are commonly higher than these in the fish which feed on them (Jackson et al., 1980; Reid and McGreer, 1981; Heit and Klusek, 1984).

Waterborne uptake cannot, however, be dismissed. If the tissues of sucker from each site are ranked from lowest to highest on the basis of Cu or Zn content, the only tissue which shows an appreciable change in rank at MAN, relative to LOK, is gill. For copper, gill goes from the lowest concentration of the eight tissues at LOK to the fourth highest at MAN. The Zn concentration in gill goes from sixth highest at LOK to third highest at MAN. While it is tempting to attribute these

changes solely to increased metal uptake by gill, it must be noted that, for Zn at least, gill can serve as a major excretory route (Hardy et al., 1987).

While dietary Cu is absorbed through both the stomach and intestine, dietary Zn is absorbed almost exclusively through the wall of the intestine (Shears and Fletcher, 1983). Although there are reports of mutual antagonism with respect to the dietary uptake of Cu and Zn (Shears and Fletcher, 1979; Knox et al., 1984), no such effect was evident here. The concentrations of both Cu and Zn increased in the anterior intestine, relative to the stomach, although the effect on Zn was more obvious. The concentration of Cu remained fairly constant throughout the gastrointestinal tract, and stomach levels exhibited no correlations with levels in digesta from the intestine or feces. Since the major pathway for Cu excretion appears to be in the bile (Evans, 1973), the input of biliary Cu into the anterior intestine, and/or absorption in the stomach or intestine, may have obscured the presence of any relationship. This is supported by the positive correlation between posterior intestinal and fecal Cu burdens.

The increased concentration of Zn in the anterior intestine is largely a reflection of the absorption of diet constituents and the binding of Zn by cuticular proteins in the exoskeletons of invertebrates (Milner, 1979; Dallinger and Kautzky, 1985). At the control site, the declining Zn concentration in the posterior intestine is reflective of continued absorption in the anterior portion of the digestive tract. This absorption of Zn after biliary input suggests some evidence for enterohepatic cycling in these fish.

Overall, the profile of metals in tissues of MAN are indicative of a population of white sucker exposed to metals within the range of homeostasis. Since this work

was completed with relatively large fish (490 to 1420 g), this finding is not inconsistent with the population effects which we previously reported for this system (Munkittrick and Dixon, 1988b). All of the direct metal effects which we reported were expressed as impacts on larval fish (Munkittrick and Dixon 1988b). The predominant impacts on adult fish appeared to be indirect, and suggested that the females were unable to accumulate sufficient energy to meet the demands of both growth and reproduction. Information available on the Manitouwadge chain suggests that benthic invertebrates are decreased in number, or absent from, deeper areas (>5 to 7 m) of the contaminated lakes (German, 1971; Pugh and Maki, 1986). The fauna of shallower areas in the contaminated lakes is dominated by chironomids and tubificids, and either lacks, or has a marked decrease in the abundance of, the mayflies, caddisflies, snails and clams characteristic of the reference lakes in the chain (German 1972; Pugh and Maki 1986; Munkittrick and Dixon, unpubl. data). This suggests that the majority of contaminant effects on Manitouwadge sucker are indirect, resulting from alterations in the available food base, consistent with the apparent homeostasis reported here.

Chapter VII

BENTHIC MACROINVERTEBRATE DISTRIBUTION

7.1 Introduction

The major phenomenon associated with the sucker population at the contaminated site is associated with the decreased growth performance of female fish. The growth proceeds at a rate comparable to the reference sites until the age of sexual maturity. After this point in time, the female fish at contaminated sites are no longer able to maintain a significant growth rate. Much of the data collected suggests that the fish do not accumulate sufficient energy to meet the demands of both growth and reproduction, although before maturation they can meet the lower demands of growth alone.

The fish also exhibit decreases in fecundity and egg size. The decreased fecundity, egg weight, larval size and increased larval developmental rate are consistent with the presence of decreases or impaired energy reserves, a hypothesis which is supported by the decreased muscle lipid reserves. To further investigate the relationship of these deficiencies with the environment at the contaminated sites, it was necessary to examine the food availability at the contaminated sites.

The findings of elevated metal residues in the stomach contents of sucker from the sites prompted a further examination of sediment and water metal contamination. The number of sampling stations and lakes was increased to expand the information available. It was necessary to examine the feeding of the suckers and food availability.

7.2 Methods

7.2.1 Sample collections

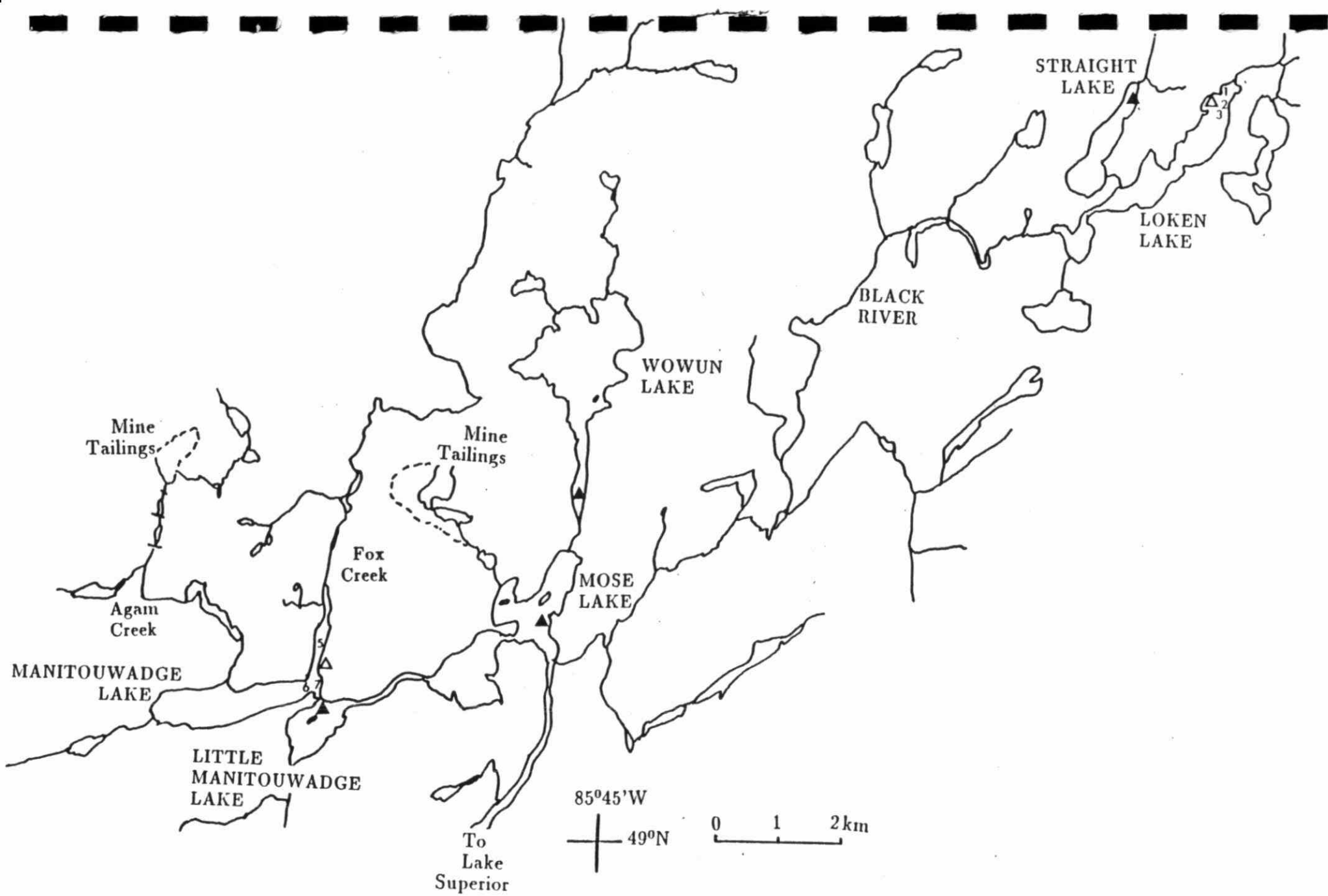
During June, 1987, collections were made to examine levels of metals in the water and sediment of lakes in the Manitouwadge chain, along with the relationship between the invertebrate community and sucker stomach contents at selected sites. Samples were collected from contaminated sites at Manitouwadge (MAN), Little Manitouwadge Lake (LMN) and Mose Lake (MOS) and from control sites on Loken Lake (LOK), Straight Lake (STR) and Wowun Lake (WOW). At MAN, samples were collected from three sites, one site consisting of highly organic matter (Fox Bay), one site with predominantly sand as the substrate and a third site with approximately 50% organic and 50% sandy substrate. The collections were duplicated at LOK (Figure 17).

Sediment and invertebrate samples were collected at depths of 2-3 m. Sediments below deeper waters were not sampled since previous work had concluded that sediments below 5 m water depth were incapable of supporting macroinvertebrate fauna (German, 1971; Pugh & Maki, 1986). The collections were made in the same areas where sucker had been previously collected.

Sediment samples were collected with a modified FBA core sampler, which collected four simultaneous cores of 5.5 cm diameter. Twenty-four samples were collected for invertebrate analysis and six samples were collected for measurement of sediment metals. Of the samples collected for invertebrate identification, only six from each site have been sorted to date.

Figure 17:

Sampling sites for sediment and invertebrate samples.. Samples were collected at depths of 2 to 3 m with a modified FBA core sampler (5.5 cm diameter).



7.2.2 Metal analysis

For metal analysis, sediment samples were immediately frozen in liquid nitrogen. For invertebrate identifications, samples were sieved on site through a 200 μm mesh bag, and the residue was placed in 10% formalin for later separation. Water samples were collected from 0.5 m depth, acidified to 1% nitric acid and analyzed by flame atomic absorption spectrophotometry.

Total non-residual metal levels were measured in the sediment samples. The samples were thawed, sieved through a 20- and 80- mesh sieve, and a 1.0 g sample was placed in 100 ml of 0.5 N HCl. The sample was shaken for 16 hr, filtered through a 0.45 μm cellulose acetate filter and the leachate was analyzed for copper and zinc content by flame atomic absorption spectrophotometry.

White sucker were collected between the hours of 1000 and 1800 at MAN and LOK since previous samples had shown that the fish would be actively feeding at this time. Fish were collected as previously described and stomach contents were placed in 70% ethanol for subsequent identification. Insects were identified to genera, and the other invertebrates were identified only to order.

7.3 Results and Discussion

Levels of copper and zinc recorded in the water at MAN and LOK were similar to those reported in 1985 and 1986 (Table 30), and values at all contaminated sites were elevated relative to those at LOK. Sediment levels of non-residual metals showed a lot of variation between sites, especially at MAN, and mean levels were 102 mg kg^{-1} copper and 1150 mg kg^{-1} zinc (Table 30). Control sites showed only background levels of copper and zinc in the water and sediments.

Table 30: Levels of copper and zinc detected in the water and sediments of lakes in the Manitouwadge chain during June, 1987. The water levels represent total, unfiltered metals and the sediment levels given are for total, non-residual metals. Levels are given as $\mu\text{g L}^{-1}$ for waterborne metals and mg kg^{-1} for sediments. Values are given with standard error in brackets, $n=6$ for all sites.

Lake	Site	Water		Sediment	
		Copper	Zinc	Copper	Zinc
MAN	1	9.8 (1.1)	231 (1)	160 (58)	2375 (762)
	2	9.5 (0.4)	235 (1)	123 (17)	833 (150)
	3	9.8 (2.3)	230 (3)	25 (8)	238 (85)
	avg.	9.7 (0.8)	232 (1)	102 (8)	1149 (328)
MOS		4.6 (1.7)	117 (3)	144 (24)	1410 (538)
LMN		9.2 (0.9)	171 (3)	44 (21)	567 (273)
LOK	1	3.2 (0.7)	7 (3)	7.5 (1.5)	31 (4)
	2	1.3 (0.9)	19 (5)	4.0 (1.1)	20 (2)
	3	1.7 (0.7)	4 (2)	22.7 (6.4)	79 (13)
	avg.	2.1 (0.4)	10 (2)	11.4 (2.9)	43 (7)
WOW		9.2 (3.7)	13 (2)	12.7 (3.5)	29 (4)

Invertebrate taxa found during sampling were similar to those reported previously by both German (1971) and Pugh and Maki (1986) (Table 31). The most striking results were the almost complete absence of mayflies (ephemeroptera), amphipods, dragonflies (odonata), stoneflies (plecoptera) and caddisflies (trichoptera) at contaminated sites (Table 32). The collections had been concentrated in the shallow near-shore areas since the previous studies by German (1971) and Pugh and Maki (1986) had reported an almost total absence of macroinvertebrates in water deeper than 5 m. In 11/15 samples collected by German in Mose Lake, no invertebrates were found, and only 5 specimens were in the remaining 4 samples. Pugh and Maki (1986) also concluded that at MAN, sediment under water more than 5 m deep was not capable of supporting a normal macroinvertebrate fauna.

The total density of invertebrates varied from $>15000\text{ m}^{-2}$ at LOK to <8000 at MAN and MOS lakes (Table 32). Chironomid species and other dipterans accounted for 78 to 96% of the total numbers of individuals at MAN, MOS and LMN, as compared to 40 to 75% at the control sites (Table 32). Although we did not find large numbers of tubificid worms in these collections, Pugh and Maki (1986) reported that the numbers of tubificids increased in deeper water at MAN.

The abundance and diversity of chironomid species varied, with contaminated sites yielding lower numbers and fewer species (Table 33). The dominant species of chironomid also changed, with the genera of *Procladius* and *Pagastiella* accounting for 80% of the chironomids (numerically) at MAN ($<18\%$ at LOK) and the genera of *Polypedilum*, *Tanytarsus* and *Cladytanytarsus* accounting for more than 60% of

Table 31: Invertebrates reported in Manitouwadge lakes, 1969-88
 (G- German, 1970; P-Pugh and Maki, 1986;U-Unconfirmed;
 *- both German & Maki; M-this study).

Site	Contaminated Intermediate Clean										
	MAN	MOS	LMN	LMS	KAG	AGN	WOW	BLA	MOR	STR	LOK
Ephemeroptera											
Caenidae											
Caenis					P	P		M	P	P	M
Emphegeridae											
Hexagenia					G	P	P	GM	P	G	M
Ephemera								P	P		
Odonata											
Anisoptera											
Libellulidae											M
Gomphidae								P			M
Corduliidae								U			
Zygoptera											
Ischnura					P						
Chromagrion					P						
Sp.								P			
Hemiptera											
Sp.		P					P				
Coleoptera											
Sp.					P						
Chrysomelidae											
Donacia								P			
Dytiscidae											M
Laccophilus			U								
Hydrophilidae											
Hydrochara					P		P				
Carabidae											M
Trichoptera											
Sp.							P	P			
Parapsyche											M
Hydropsychidae					P	P		U			
Cheumatopsyche			G								
Leptoceridae											
Oocetis		M		G			M			M	M
Phylocentropus					P				G		
Platycentropus							P				
Polycentropus		P		G							
Molannidae											
Molanna							P	U			
Limnephilidae											
Limnephilus		P			P			P			
Astenophylax							P				

Table 32: Density of benthic organisms (# m⁻²) collected during June, 1987. Proportions (numerical) of benthos collected are shown in brackets.

Group	LOK			WOW	STR	MOS	LMN	MAN		
Site	3	2	1					6	5	7
Amphipoda	560 (3.3)	3290 (10.3)	2520 (8.6)	280 (2.6)	6650 (72.5)	140 (2.9)				
Ephemeroptera	560 (3.3)	700 (2.2)	504 (1.7)	1120 (10.5)				70 (1.1)		
Odonata		70 (0.2)	84 (0.3)							
Coleoptera			168 (0.6)							
Megaloptera				140 (1.3)						
Plecoptera		140 (0.4)								
Trichoptera	70 (0.4)	280 (0.9)		70 (0.7)	70 (0.8)					70 (0.8)
Diptera	12670 (75.1)	21700 (67.7)	11928 (40.8)	6370 (59.4)		3920 (81.6)	8470 (96.0)	5320 (83.5)	2800 (78.4)	8120 (95.9)
Planaridae		70 (0.2)	168 (0.6)							
Bivalves	1190 (7.1)	1190 (3.7)	2100 (7.2)	140 (1.3)	630 (6.9)	140 (2.9)	70 (0.8)		140 (3.9)	
Gastropods	350 (2.1)	1470 (4.6)	4704 (16.1)	2030 (19.0)	210 (2.3)					
Hydracarina	140 (0.8)	280 (0.9)	1932 (6.6)	70 (0.7)	70 (0.8)	140 (2.9)		70 (1.1)		
Annelida*	1330 (7.9)	2870 (9.0)	5292 (18.1)	490 (4.6)	1540 (16.8)	490 (10.1)	280 (3.2)	910 (14.3)	630 (17.6)	280 (3.3)
Total	16870	32060	29232	10710	9170	4830	8820	6370	3570	8470

* for this table annelida includes oligochaetes, nematomorphs and nematodes

Table 33: Abundance of chironomid species.

Species	LOK 1	2	3	STR	WOW	LMN	MOS	MAN 5	6	7
Polypedilium simulans	5460	3864	420	168	588	-	-	-	-	-
Tanytarsus	1596	5124	2100	252	252	336	252	-	-	84
Cladotanytarsus	840	5796	1554	-	1092	1008	252	84	-	1344
Procladius	1596	1764	3864	1092	2100	1512	1260	756	2268	1764
Microtendipes	84	84	1764	336	-	-	84	-	-	252
Dicrotendipes	420	294	714	756	252	-	252	84	-	84
Polypedilium sp.	168	420	840	-	-	-	-	84	-	-
Pseudochironomus	-	966	126	-	84	-	-	-	-	-
Thienemanninigia -gp.	84	84	294	84	-	-	-	168	-	-
Clinotanypus	84	210	126	-	168	-	-	84	-	-
Ablabesmijia	168	84	126	-	168	-	-	336	-	-
Epoicladus	-	210	126	-	252	-	-	-	-	-
Parakiefferiella	-	210	126	84	84	588	252	168	84	168
Cryptotendipes	252	84	-	168	1008	504	252	84	-	1008
Pagastiella	168	126	84	-	756	5124	1176	1176	2940	4452
Parachironomus	84	84	126	84	-	-	-	-	-	-
Nilothanna	-	84	-	-	-	-	84	84	-	-
Paratendipes	-	-	126	-	168	-	-	84	-	-
Phaenopsactra	84	-	-	-	-	-	-	-	-	-
Cryptochironomus	-	-	-	-	84	-	-	-	-	-
C. isocladius	84	-	-	-	-	-	-	-	-	-
Hydrobaenus	84	-	-	-	-	-	-	84	-	-
Stempellinella	-	-	-	168	-	-	-	-	-	-
Total	11256	19488	12516	3192	7056	9072	3864	3276	5292	9156

individuals at LOK (<4% at MAN) (Table 34). The significance of these changes are not known.

Table 34: Relative abundance (%) of dominant chironomid species at LOK and MAN sites. Estimates were pooled as an average of the collections made at three sites, average density was 5591 chironomids m⁻² at MAN and 14409 m⁻² at LOK.

Site	MAN	LOK
Species		
<i>Polypedilium simulans</i>	0	22.5
<i>Tanytarsus</i>	0.5	20.4
<i>Cladytanytarsus</i>	2.9	18.9
<i>Procladius</i>	28.5	16.7
<i>Microtendipes</i>	1.5	4.5
<i>Pagastiella</i>	51.1	0.9
<i>Parakiefferiella</i>	2.5	0.8
<i>Cryptotendipes</i>	6.5	0.8
Total of numbers recorded	93.5	85.5

There was a marked decrease in diversity of invertebrates identified at contaminated (MAN, MOS) and intermediate (LMN, LMS, Kaguinu (KAG) and Agonzon (AGN)) lakes relative to controls (Table 35). The density of invertebrates at contaminated sites was 60% lower than at control sites, and the number of genera recorded declined more than 50%, in shallow water (Tables 32, 35). A major difference detected between sites was the absence of beds of unionid clams at contaminated sites. Sediment collections at all lakes were made adjacent to emergent macrophytes in water of 2 to 3 m depth. At all control sites large beds of *Anodonta grandis* and *Lampsalis siliquioidea* clams were visible at these sites. Active searching of sandy, shallow areas in all contaminated lakes failed to detect any clams, although German (1971) reported finding empty *L. siliquioidea* shells in Mose Lake.

An analysis of stomach contents of white sucker showed that stomach contents were generally reflective of the food availability (Table 36). Numerically, 60% of the organisms identified in LOK stomachs were dipterans, while this group constituted 61% of the invertebrates collected in sediment samples. The largest difference in occurrence at LOK was for the mayflies (ephemeroptera) which accounted for only 2% of the benthic organisms, but more than 20% of stomach contents. This may have been related to the proximity of sampling time to the time of emergence of the mayflies. Large numbers of emergent mayflies were found at all control sites, and were

Table 35: Numbers of insect genera recorded 1969-1987 from contaminated (MAN, MOS), intermediate (LMN, LMS, KAG, AGN) and clean (STR, LOK, BLA, MOR) sites from the Manitouwadge chain.

Order	Contaminated	Intermediate	Control	Total
Odonata	0	2	4	6
Ephemeroptera	1	2	3	3
Plecoptera	0	0	1	1
Hemiptera	1	0	1	1
Megaloptera	1	1	1	1
Coleoptera	1	2	2	3
Trichoptera	3	5	7	10
Diptera	13	6	16	16
TOTAL	20	18	35	41
Others				
Amphipoda	0	0	1	1
Annelida (Hir)	1	0	3	4
(Oli)	2	1	5	5
Pelecypoda	2* no unionids	2	4	4
Gastropoda	1	2	6	8
Hydracarina	1	1	1	1
TOTAL	27	24	55	64

not seen at any contaminated sites, except for MOS where a few individuals were seen. The single mayfly collected at contaminated sites in bottom samples (Table 32) was a member of the genus *Caenis*, while the dominant species at control sites was the much larger *Hezagenia*.

At contaminated sites, sucker stomachs yielded an average of 49.8 organisms, relative to 7.8 at LOK (Table 36). The bulk of this difference was made up by chironomids, MAN sucker had an average of 29.8 chironomids relative to 4.4 at LOK. The MAN sucker also appeared to be preferentially feeding on trichopterans, since their density was much higher in the stomach samples than in the bottom samples. Pugh and Maki (1986) reported that trichopterans were more common at MAN sites which contained gravel, and it is probable that the relative increase in frequency in MAN guts is a reflection of an increased foraging effort at the contaminated sites. A single dragonfly nymph was also encountered in a MAN stomach contents although our bottom samples failed to detect their presence. A final worthy note on the MAN sucker stomach contents was the relatively high occurrence of cladocerans and copepods, which were not found at all in LOK stomachs.

The density of organisms found in our benthic samples was much higher than those reported by Pugh and Maki (1986) or German (1971) (Table 37). This is probably due to an increased efficiency of our sampling methods, Pugh and Maki used a Ponar grab, while German had used an Ekman dredge. The species occurrence is similar.

Table 36: Stomach contents of white sucker collected in June of 1987. Numbers are expressed in terms of total food organisms, average number of organisms per stomach and percentage (numerically) of diet.

Site	MAN (n=12)				LOK (n=10)			
	Total Number Recorded	Average % Per Stomach	Actual Sediment Frequency		Total Number Recorded	Average % Per Stomach	Actual Sediment Frequency	
Chironomids	357	29.8	60.0	88.8*	44	4.4	56.4	61.2*
Ceratopogonids	114	9.5	19.1		4	0.4	5.1	
Trichoptera	62	5.2	10.5	0.2	4	0.4	5.1	0.1
Ephemeroptera	-	-	-	0.4	17	1.7	21.8	2.4
Odonata	1	0.1	0.2	-	-	-	-	0.2
Oligochaeta	1	0.1	0.2	10.9**	5	0.5	6.4	11.8**
Hydracarina	2	0.2	0.3	0.3	-	-	-	2.2
Platyhelminthes	-	-	-	-	1	0.1	1.3	0.3
Bivalva	3	0.2	0.5	0.7	-	-	-	6.0
Cladocera	54	4.5	9.1		3	0.3	3.8	
Copepoda	2	0.2	0.3		-	-	-	
Total	596	49.8	100.2		78	7.8	99.9	

*includes chironomids and ceratopogonids

** includes nematodes and oligochaetes

Table 37: Comparison of densities found in this study (number per m²) with those reported by German (1971) and Pugh and Maki (1986).

Group	MAN			LOK		WOW	
	This Study	Pugh & Maki (1986)	German (1971)	This Study	Pugh & Maki (1986)	German (1971)	This Study
Amphipoda				2123	197		280
Ephemeroptera	23		rare	591	45.5	rare	112
Odonata				51			
Hemiptera					3.8		
Megaloptera					3.8		140
Coleoptera				56			
Diptera	5410	68.2	11/ft ²	15432	136.2	31/ft ²	6370
Trichoptera	23	3.8	rare	117	51.3		70
Hydracarina	23			784	15.2		70
Planaridae				80			
Bivalves	47			1493	11.4	rare	140
Gastropoda				2175	3.8	rare	2030
Oligochaeta	537	288	rare	3164	113.7	rare	460
Nematodes	47			519			
# taxa	6	5	5	11	22	7	9
#/m ²	6090	360	12/ft ²	26585	569	37/ft ²	1071
		site 4 2.8 m depth .052 m ² Ponar grab	9x9 in Ekman dredge				

The changes in diversity and density of macroinvertebrates are consistent with the data documenting changes in fish growth and reproduction. Similar decreases in abundance and diversity have been reported previously for damaged aquatic ecosystems (Roback, 1974; Table 38), but it is very difficult to make many generalizations. The fact that the lakes are closely linked in a continuous chain suggests that all lakes should have an equal potential to support fauna (invertebrate and vertebrate), and the correlation of changes in this potential with the runoff from mine tailings suggests a direct relationship.

Although we did not sample Little Mose Lake (LMS), Pugh and Maki (1986) reported that the fauna included ephemeroptera, trichoptera, odonata, coleoptera and gastropoda, which were all lacking or decreased at contaminated sites. The position of Little Mose Lake between LMN and MOS also provides evidence for a correlation of the changes with the increased metal burdens. Increased metal burdens were associated with the stomach contents of sucker from the contaminated sites (Miller et al., 1988). It is very clear that MAN sucker must commit a great deal more energy to finding and eating food than the fish at LOK.

Table 38: Numbers of species of insects present in clean and damaged ecosystems (Roback, 1974). Values are given for mean number of species, range of occurrences, number of times the order was absent and times infrequently sampled (< 2 sp.) in either clean (n=13) or damaged (n=10) environments. Values in brackets show % of time species were absent or present in low numbers in damaged environments.

Order	Clean system (n=13)				Damaged system (n=10)			
	Mean	Range	Absent	<2 sp.	Mean	Range	Absent	<2 sp.
Odonata	8	3-15	0	0	4	0-7	2	1 (30%)
Ephemeroptera	8	4-15	0	0	2	0-7	4	2 (60%)
Plecoptera	3	0- 7	3	2	<1	0-1	7	2 (90%)
Hemiptera	5	2- 7	0	0	1	0-5	5	3 (80%)
Megaloptera	2	0- 3	2	3	<1	0-3	7	2 (90%)
Coleoptera	11	3-17	0	0	3	0-10	3	1 (40%)
Trichoptera	5	1- 8	0	1	2	0-6	3	4 (70%)
Diptera	12	6-17	0	0	4	0-13	1	2 (30%)
Total	31-71				2-43			

Chapter VIII

PISCES FRAMEWORK

8.1 Introduction

In-depth analysis was successful at documenting changes in sucker growth, reproduction and larval survival associated with exposure to metals. However, the assessment was time-consuming, very expensive and still requires detailed follow-up analysis. The use of such an evaluation framework on a routine basis would be prohibitively expensive.

It should be possible to develop a simpler, cost-effective approach which could allow preliminary indications of impact. This chapter describes the adaptation of such a framework for our purposes.

8.2 Relevance

An ecosystem health assessment framework would be valuable in the assessment of areas identified by current surveillance techniques as being of high-risk for the impact of stressors or in testing for site-specific criteria. The current water quality criteria play a key role in identifying those areas. Changes in the performance of individuals should be evident at levels of stress below those affect-

ing survival. These changes should be reflected in the overall characteristics of populations. There is a limited control of population size and community interactions associated with energy flow, nutrient levels and habitat availability.

Fish populations normally produce a surplus of offspring and rely on ecosystem conditions for the regulation of survival. The basis for the compensatory concept is that a change in survival initiated by density-independent fluctuations in environmental conditions is corrected for by ecosystem-related, density-dependent, compensatory mechanisms. Although little is known about the identity of such factors, density-dependent changes in population characteristics have been documented (George, 1977; Healey, 1978; Goodyear, 1980; Colby, 1984; Wedemeyer et al., 1984). Effects can be direct, acting on growth, reproduction or survival, or can be indirect, operating through effects on predators or forage food.

8.3 Rationale

The main assumption of this framework is that fluctuations in the birth or death rates, or the availability of food or habitat resources, will result in a change in population size or biomass. Such changes should be identifiable, and ideally will provide information on the impact site associated with the change. Compensatory changes are well documented at the population level, and include a change in yield (Klontz, 1984), a change in population structure with respect

to size distribution, age-class strength, growth rate or sex ratio (George, 1977; Colby, 1984), recognizable lesions or deformities which may be increased in polluted areas (Sonstegard and Leatherland, 1984; Munkittrick et al., 1985), and behavioural changes (Klontz, 1984).

The response of fish populations to a stressor should be similar whether the stressor is associated with fishing or chemical stress. Alterations in population characteristics due to fishing pressure have been identified, and include those associated with growth rate, mortality rate, size at age, age at maturity, mean age, longevity, fecundity, gonadosomatic index, condition factors, sex ratio, catch per unit effort (CPUE) and yield, particle size and species composition of aquatic communities (Colby, 1984). There have been attempts to simplify phenomena associated with many of these variables into fisheries models. Vaughan et al. (1984) discuss the use of these models for the assessment of the effects of fishing on populations or stocks of fish. There are several major disadvantages when trying to adapt these models for use in the assessment of toxicant impact on aquatic ecosystems. The majority of these problems lie in the assumptions required by the models or in the type of data required for the manipulations. Some models require data from population harvests collected over a number of years, assume that life history parameters are independent of time and density, and can not differentiate changes in mortality from effects on growth or reproduction

(Vaughan et al., 1984). Many models do not permit accurate estimation of population size, and Levin (1982) concluded that "mathematical models hold great potential as predictive tools, but only after the relevant equations are accurate enough to justify confidence."

Any systematic investigation will require sound hypotheses and statistical sampling designs (Table 39). Several comprehensive testing regimes have been successfully used to assess ecosystem health, and have included the monitoring of sediment and water contamination, primary production, chlorophyll levels, enumeration of benthos and measurement of muscle contaminants in fish (Pearce, 1984), and combined bioassay testing with an assessment of reproductive impairment, larval survival and growth (Chapman et al., 1985). Both programs were successful in identifying changes in the affected fish populations in terms of growth, reproduction and/or larval survival, but are prohibitively expensive to use routinely as part of a general surveillance program.

In laboratory testing, growth, reproduction and larval survival are the most toxicant-sensitive aspects of a fish's life history (see introduction for references). All three responses incorporate the fish's experience over a considerable period of time into factors measureable with a minimum of equipment and expertise. Population data based on maturity, fecundity and year-class strengths offer insight into population fluctuations over the past several years. The underlying principle of this framework is that a population of

Table 39: Appropriate sampling protocol (adapted from guidelines proposed by Green, 1979; Donaldson and Scherer, 1983; NRCC, 1985).

1. Choose a suitable control population for comparison with the altered site. For results to be of any value, the characteristics of the threatened environment must be duplicated as closely as possible, especially with respect to lake size, location, habitat potential (MEI) and with standardization for as many biotic and abiotic modifying factors as possible. Set up study to look for differences in growth or reproductive potential between the groups.
2. Randomly take preliminary samples to evaluate sampling design, heterogeneity of environment and extent of contamination. Make sure that the samples are coming from the portions of the population that are required, i.e. that the sampling sites are not restricted to nursery areas, etc.
3. Estimate the sample size requirement and decide if subsampling is required for different areas. and examine samples for normality and heterogeneity.
4. Evaluate factors representative of the growth and reproductive potential .

<u>Population Indicators</u>	<u>Reproductive Indicators</u>			
length	Males	Females	Post-fertilization	Post-hatch
weight	spermatogenesis	egg size	water hardening	survival
age	spermiogenesis	fecundity	fragility	deformities
condition factor	timing of development	atresia	adhesiveness	behavior
growth rate	behavior	histology	egg size	heart rate
sex ratio	GSI		buoyancy	yolk utilization
neoplasm incidence	Performance:		Embryonic development	developmental rate
structural deformities	milt quantities	spawning success	pre-hatch mortality	growth
contaminant burdens	behavior	behavior	time to hatch	acclimation and
parasitic infections	timing, efficiency	timing	size at hatch	tolerance response
	egg survival	egg quality	size of yolk sac	
		fertility		

5. Use biochemical analysis to isolate the impact points.

fish found to be growing, reproducing and surviving within the "limits" of a comparable control population shall be considered free from detrimental effects of contaminant exposure. Obviously this approach is very simplified, but the purpose of this framework is to "develop a procedure which is better than trial and error where no method of arriving at an exact solution is known" (Silvert, 1981).

8.4 Monitoring framework

There are three steps in identifying the effects of contaminant stress on ecosystems

1. define the extent of contaminant exposure
2. identify the population size and the presence of other stressors
3. if there are no changes associated with the exposure, examine the entire community (Wedemeyer et al., 1984).

Population-level effects of fishing and contaminant stress should be indistinguishable and Colby (1984) has identified five patterns of population response to fishing pressure, which can be adapted for examining the effects of chemical stressors. The exposure of a large portion of the adult population to acutely lethal chemical levels would be analogous to the fisheries exploitation of a new stock or a relatively non-selective harvest of the adult population. In either case, the result is a decreased population size and therefore an increased food availability (Table 40). Such a change in the ecosys-

tem should result in an increased growth rate of survivors, a decreased age at maturation and an increased fecundity and condition factor due to increased food resources (Table 41). The decimation of the adult population would result in a decreased mean age of the population and a shift in the size distribution towards younger fish.

Similar responses can be identified for several other alterations in the ecosystems, such as a reproductive failure or elimination of food resources (Table 40,41). Population characteristics do not act independently within a response pattern, and characteristics can be grouped based on common mechanisms. Age at maturation data, fecundity data and growth rate responses occur at the same time since all three are related to food availability. Similarly, changes in mean age and age distribution data occur simultaneously, and reflect direct responses of the populations to the stressors.

It is not necessary to examine all ten parameters and responses can be separated into the five response patterns based on an examination of mean age, fecundity and condition factor (Table 42). If data on one parameter is not available, then substitutions are possible. For example, fecundity data could be replaced by growth rate or age to maturation data, since changes in the three parameters are usually simultaneous.

Table 40: Resource and population consequences of chemical-induced alterations in ecosystem quality.

Response Pattern	Chemical-Induced Stress	Equivalent Non-Chemical Stress	Effect on Population	Resource Consequences	Initial Population Consequences
I	Acutely lethal spill away from nursery areas or a response to chronic mortalities	Exploitation by fishery	Reduced abundance of adults	Increased food availability	Increased growth rate of survivors
II	Spawning failure or increased larval mortality (reproductive problems, contamination of spawning areas or yolk-associated burdens)	Loss or disturbance of spawning grounds	Reduced abundance of young fish	Decreased use of food and habitat resources	Progressive shift in age distribution towards older fish
III	Body burden problems or restricted food supply decrease energy flow through juveniles	Size-selective mortality of small fish induced by habitat change or loss of food supply	Reduced abundance of young fish	Decreased replacement of adult fish	Progressive shift in age distribution, decline in condition of fish
IV	Predator removal by chemical events or chronic food availability problems	Overstocking of fish or overexploitation of predators controlling population	Increased population size or increased competition for food	Decreased food availability	Decreased growth rate
V	Habitat restriction, reduction or loss of food supply	Habitat destruction or introduction of exotic species	Increased food competition	Food web restructuring	Decrease in feeding efficiency

Table 41: Population characteristics of the five response patterns (adapted from Colby, 1984). The response shown is the relative response of the contaminated population to the control population. A relative increase is shown by a plus sign, a decrease by a minus and the absence of a change by a zero.

Pattern	Mean Age	Age Distribution	Growth Rate	Condition Factor ¹	Age at Maturation	RLS ²	Fecundity	Egg Size	CPUE ³	Population Size
I	-	-	+	+	-	-/0 ⁴	+	-/+	-	-
II	+	+	0/+	0	0	+	0	0	-	-
III	+	+	-	-	+	0	-	-/0	-	-
IV	0	0	-	-	+	-	-	-	+	+
V	0	0	-	0	+	-	-	-	-/0	-/0

¹Condition factor (k)= $100(\text{wt}/\text{length}^3)$

²Reproductive life span = mean age - (age to maturation)

³Catch per unit effort

⁴Change can vary with amount of stress

Table 42: Separation of response patterns based on the relative changes in mean age, fecundity and condition factor (k) relative to a comparable group of control fish.

Response Pattern	Mean Age	Fecundity ¹	k	Reasoning
I	-	+	+	Elimination of adults decreases mean age; resulting increase in food availability increases growth, fecundity and k
II	+	0	0	Elimination of recruitment increases mean age, survivors may show no change in fecundity, possible increase in k
III	+	-	-	Poorer recruitment increases mean age; poorer food supply decreases fecundity and k
IV	0	-	-	Increased population size decreases food availability, fecundity and k
V	0	-	0	Change in feeding habits and increased competition decreases feeding efficiency and decreases fecundity ²

¹Could be substituted by age to maturation or growth rate

²If problems were to continue, fecundity and k may decrease (pattern IV), further stress would decrease the population size with a resultant increase in mean age (pattern III). If problems were to continue, the population would decline to a size that the food could support, resulting in an increase in fecundity and k to control levels (pattern II). If population continued to decline, there would soon be more food than required, and fecundity and k would increase, with a decrease in mean age (pattern I).

8.5 A priori sampling limitations

Ideally, for the best results comparisons should be restricted to within a certain age-class for a single sex of fish. Under ideal conditions, the response of the male fish should be separated from the females, since the response may differ substantially, especially when subjected to food-limitation stresses. For the purpose of this thesis we will try to restrict comparisons to within sex, but limitations of data sets available in the literature limit comparison in too much detail.

Extreme caution must be used when setting up the experimental design to choose a control site with similar habitat, carrying capacity and exploitation stresses. If data on any single parameter is not available, it is possible to substitute data from a similar response parameter, i.e. growth rate data could be substituted for fecundity data. Male fish could be easily compared by substituting growth rate or age to maturation data for fecundity data. Other characteristics, such as the sex ratio, are of unknown importance in suckers, but may have significant meaning for other species such as coregonids (George, 1977). The failure of a response pattern to follow all response characteristics (Table 41) would suggest areas for further study. After fitting a response to a particular pattern based on characteristics of age, fecundity and condition factor, a larger data set can be used to compare population characteristics to the overall response pattern. Places where major differences between

expected and observed responses occur signify areas requiring follow-up study.

The simplicity of the approach ignores the time frame involved in the response of fish populations and adaptations of the ecosystem. However, this may not compromise the ability to trace impact. An increase in waterborne metals may eliminate sensitive macroinvertebrates, causing the suckers to depend on alternate, less-efficient food sources. The decreased feeding efficiency could lead to a decrease in reproductive effort, and a type V response pattern would be detected. After a prolonged duration of decreased feeding efficiency, the condition of the fish would likely decline and the response pattern would be detected as type IV. Further stress would decrease the population size with a resultant increase in mean age, associated with a decreased reproductive success; changes would now be detected as type III. If problems were to continue further, the population would continue to decline until it reached numbers which could be supported by the food base. At this time the fecundity and condition of the fish should return to control levels, but the persistence of an increased mean age would be classified as a type II response. If the population size were to continue to decline, there would soon be an excess of food, resulting in an increased growth rate, fecundity and condition factor and a decline in mean age, suggesting a type I response.

8.6 Application of the framework

The framework has been abbreviated as PISCES (Population Indicators of Sublethal Contaminant effects on Suckers). Providing that the selection of a control site is done carefully, the PISCES approach should be applicable to any ecosystem. Within Canada, two very different applications are immediately obvious.

1. Many lakes exhibiting water quality parameters in excess of current guidelines or criteria are small, isolated lakes in areas associated with primary resource extraction. In many cases, uncontaminated lakes of similar size and potential productivity are situated nearby and comparison of stocks would be relatively easy.
2. There are areas within large bodies of water such as the Great Lakes where contaminant levels are elevated locally, especially in heavily industrialized regions. Selection of a control site and identification of exposed fish in these cases can be much more difficult, but several possibilities exist for getting around the problem. Data can either be compared
 - to a historical data set from a time preceding the input of contaminants
 - or the population could be split into exposed and non-exposed individuals. In this case the response pattern would have to be interpreted carefully since the comparison would be within a population and not between populations.

The PISCES framework can be applied in two different ways. The collection of a small number of adult females could be analyzed for age, fecundity and condition factor, and compared to a small number of control females using the abbreviated separation scheme (Table 42). This would provide a rapid estimate of the direction for future assessment. The complete response pattern can also be applied to existing data sets for comparison. There are a few studies which contain a sufficient data base to allow us to test the utility of the PISCES approach.

8.7 Case histories

8.7.1 Mixed metal atmospheric fallout

A base metal smelter located near Flin Flon, Manitoba, was associated with an increased deposition of Zn, Cd, Pb, As and Cu (Van Loon and Beamish, 1977; Franzin et al., 1979; Franzin and McFarlane, 1981b) on lakes in the vicinity of the Saskatchewan-Manitoba border (approx. 55°N, 102°W). Elevations of copper and zinc were very similar to those found in the Manitouwadge lakes (13-15 and 245 ug l⁻¹ respectively) but the lakes also exhibited increased cadmium levels (0.6 ug l⁻¹; McFarlane and Franzin, 1978). McFarlane and Franzin (1978, 1980) and Franzin and McFarlane (1981a) examined the response of sucker populations to the increased metal levels.

McFarlane and Franzin (1978) reported that the suckers exhibited decreased mean age, increased growth, increased fecundity, earlier

maturation, reduced spawning success and reduced larval and egg survival, smaller egg size and decreased longevity relative to controls (Table 43). They also suggest that there was a decreased recruitment of younger fish reflected in the marked decrease in CPUE of juvenile suckers. The authors suggested that the eggs and young were the age groups most severely stressed (Franzin and McFarlane, 1980). The decreased reproductive efficiency would be expected to yield a type II response pattern, due to the decrease in the number of young fish entering the population. However, continued reproductive impairment over a period of years would eventually result in a decreased mean age as the older fish died off. This sucker population appears to be showing a classic, density-dependent compensatory response to the elimination of a large portion of the population. The changes in population parameters exhibited by these fish suggests that the population was exhibiting a classic type I response (Table 43). The decreased spawning success and absence of older year-classes suggests a direct effect on the adults, or the persistence of adverse conditions for a long period of time. This is supported by the history of atmospheric deposition of metals in the area.

Based on age, fecundity and condition factor, the population is thought to be exhibiting a type I response pattern. By comparing the expected and observed responses in the parameters (Table 43), we can look for areas which do not coincide. The only parameter which does not match up is the change in condition factor. The expected

Table 43: Summary of the response of a white sucker population to atmospheric deposition of heavy metals. The waterborne concentrations of copper (13 ppb), zinc (245 ppb) and cadmium (0.6 ppb) were elevated at Hamell Lake.

Site	Mean Age	Age Distribution	Growth Rate	Condition Factor	Age at Maturation	RLS	Fecundity	Egg Size	CPUE	Population Size
Hamell	4.27	maximum 9 yr	increased length at age	no change ³	3.5	0.8	34000	1.74	.00193	decreased
Thompson	6.22 ^{1,2}				5 ⁴	1.2 ⁵	19250 ⁶	1.82 ²	.00397 ^{2,7}	
<i>Observed Change</i>	-	-	+	0	-	-	+	-	-	-
<i>Pattern I</i>	-	-	+	+ ³	-	-/0	+	-/+	-	-

¹Includes both male and female fish

² $p \leq 0.05$

³No difference in length and weight regressions with age, assumed that k was equal

⁴At Hamell Lake, fish matured at 3-4 y, most spawners were 5-7 y
Thompson Lake, matured at 5 y and most spawners were 7-8 y

⁵Reproductive life span = mean age - (age to maturation)

⁶Estimated from fecundity, length and age regressions at age = 6 y

⁷(fish $\text{hr}^{-1} \text{m}^{-2}$) over 5 nights

response is an increased k due to the increased availability of food, but the population did not exhibit a significant change. It should be noted that the estimates of k had to be extrapolated from length, weight and age regressions, but the absence of an increase may only mean that the food availability was not sufficient for both an increase in reproductive effort and an increased k . This can also be seen in the decreased egg size at Hamell Lake. At the present time, the egg size change associated with a type I response has not been well defined and it may depend upon the food abundance at the site. The factors governing the increased fecundity associated with this type of response are not well known and the relative changes in egg size seem to be variable.

The conclusions associated with a type I response are that a large portion of the population has been eliminated and this is the obvious area requiring follow-up study.

8.7.2 Acidification

Trippel and Harvey (1987a,b) examined white sucker populations in acid-stressed and control lakes within south-central Ontario. The study was looking for evidence and information on the decreased abundance of fish populations commonly associated with acidified environments. They documented a decreased mean age at acidified sites, which strongly suggests a type I response. Increases in growth rate and condition factor, and decreases in reproductive life span and population size are also suggestive of type I response patterns

(Table 44). The authors suggested that the increased growth was a response to decreased population size and increased food abundance (Trippel and Harvey 1987b). The change in fecundity was not as large as would be expected and the only characteristic which did not fit the type I response was the increased age to maturity, which was opposite to the shift predicted by increased food availability and growth rate of fish (Table 44).

The brief reproductive period evident in the population was associated with a later age at maturation and a decrease in mean age (Trippel and Harvey, 1987a). The authors suggested that "delayed maturity under conditions of physiological stress would make possible a single, relatively large reproductive effort, prior to the high postspawning mortality" (Trippel and Harvey, 1987a). The delayed maturation may be associated with factors other than food availability and size of the fish. Walleye (*Stizostedion vitreum*) have been shown to experience spawning failure in lakes and reservoirs in the Southern United States despite an abundance of food and rapid growth (Colby and Nepszy, 1981). The failure in that case was believed to be associated with the absence of cooler water temperatures during the winter period. Among suckers in acidified environments, it has been suggested that calcium may be limiting reproductive performance (Beamish et al., 1975), although the calcium levels were similar to those found in this study. It is possible that delayed maturation in these fish may be associated with a delay in the accumulation of suf-

Table 44: Summary of the response of white sucker populations to acidification (Trippel and Harvey, 1987a,b).

Site	Mean Age	Age Distribution	Growth Rate	Condition Factor	Age at Maturation	RLS	Fecundity	Egg Size	CPUE	Population Size
Acid ¹	4.54 ³	?	increased	1.45 ⁴	3.80 ⁵	0.76	30750 ⁷	214 ⁸	2.4 ⁹	decreased
Control ²	5.52			1.36	1.98	3.5	28905	198	15.2	
<i>Observed</i>										
<i>Change</i>	-	?	+	+	+*	-	+*	+	-	-
<i>Pattern</i>										
<i>I</i>	-	-	+	+ ⁴	-	-/0	+	-/+	-	-

¹Deep acid lakes only (George, Crosson and Chub)

²Deep circumneutral lakes only (Red Chalk and Bigwind)

³Average of all lakes for females only, male values were 4.34 and 5.55

⁴Calculated from length and weight data, values for males were 1.45 and 1.33

⁵Data for females only, for males, values were 3.05 and 1.75

⁶For males, values were 1.3, 3.8

⁷Data for George and Crosson only (n=19), for control, n=31

⁸Dry weight of 100 ova

⁹Number of suckers per night

ficient resources for spawning. Similar phenomena are found in many Northern species of fish which alternate spawning years due to a shortened growing season (Dutil, 1986). This obviously represents an area warranting further study.

8.7.3 Radionuclide mining waste

This study investigated the effect of elevated radionuclides associated with uranium mine tailings effluent on lakes in Northern Saskatchewan (59°30'N, 108°35'W). Both whitefish and white suckers were examined for impact (Swanson, 1982, 1983, 1985) and compared with fish collected from two control lakes. Comparison in the text of the report (Swanson, 1982) was with suckers from Fredette Lake, and suggested that fish at the contaminated site (Beaverlodge Lake) were dominated by older, smaller suckers which exhibited a decreased growth rate and fecundity (Swanson, 1982). The decreased fecundity and growth suggests that the response is either type III, IV or V. The increased mean age suggests a type III response to a reduced abundance of young fish. Unfortunately, all of the data required for a full evaluation is not available for these fish within the report, and data from another control population (Milliken Lake) can be calculated from information in the appendices (Swanson, 1982) (Table 45).

The data on mean age, fecundity and condition factor matches up with a type III response pattern, and the limited data which are available are consistent with this finding (Table 45). The only

Table 45: Summary of the response of a white sucker population to radionuclide waste (Swanson, 1982).

Site	Mean Age	Age Distribution	Growth Rate	Condition Factor	Age at Maturation	RLS	Fecundity	Egg Size	CPUE	Population Size
Beaverlodge (contaminated)	14.25 ¹	shift to older	decreased ²	1.72 ¹	? ³	? ³	24090 ¹	1.40 ^{1,4}	no change ⁵	decreased
Milliken (control)	13.83			1.82			70875	1.79		
<i>Observed Change</i>	+	+	-	-			-	-	0 ⁵	-
<i>Pattern III</i>	+	+	-	-	+	-	-	-/0	-	-

¹Calculated from data in appendices of Swanson (1982), combined male and female

²Comparison to Milliken Lake, related to differences in food supply

³Not enough data available

⁴Wt (g) of 500 eggs

⁵But compared by Swanson (1982) with whitefish

parameter which does not follow the expected response is the absence of a change in CPUE. Swanson (1982) based this conclusion on a comparison of sucker and whitefish catch records. A type III response pattern is consistent with an increased mean age associated with poor recruitment and a decrease in fecundity and k associated with a poor food supply. The study concluded that changes in the fish populations within the contaminated lake were due to a decreased food abundance and a spawning failure associated with aluminum precipitation from the tailings effluent (Swanson, 1982).

8.7.4 Great Lakes examples

Data on Great Lakes fish populations are difficult to compare due to the problems involved with selecting a comparable control population with similar exploitation pressures. Alterations in the Great Lakes ecosystem have occurred with exploitation, habitat alteration and species introductions. Correlation of any contaminant events in the Great Lakes with alterations in species abundance may be impossible due to the enormous numbers of contaminants present. However, this may not mean that evidence of adverse effects could not be detected. The system under analysis must be kept relatively small so that sites can be duplicated in control sites as closely as possible. A thorough discussion of sentinel evaluation in the Great Lakes can be found in Ryder and Edwards (1985). Several examples dealing with white suckers are available, but the data sets are far from complete for our purposes.

8.7.4.1 Sea lamprey predation in Lake Huron

Henderson (1986) compared data collected on white sucker populations from South Bay (Lake Huron) from 1949 to 1969 with more recent data collected from 1970 to 1984. Prior to 1970, the white suckers experienced a high prevalence of sea lamprey (*Petromyzon marinus*) wounding and predation, associated with the decreasing abundance of suitable lake trout for the lamprey (Coble, 1967). The wounding was evident on suckers larger than 32 cm in length and wounding frequency increased with increasing length. During the time periods when suckers were heavily predated upon, the suckers exhibited a decreased maximum age and population size, and increased mortality rates (Henderson, 1986). After predation decreased, the maximum age of the fish increased, although there was no correlation of this with year-class strengths.

Unfortunately, data on fecundity, age to maturity or growth rate of the suckers during the early predation period is not available. Despite the absence of a complete data set, it is evident that the use of a historic data series of year-class strengths, maximum age and CPUE was effective at documenting population-level changes in sucker communities in Lake Huron. In this case, a historical data set provided the control for comparison, eliminating a search for a comparable control population.

8.7.4.2 Neoplasia in Lake Ontario suckers

As part of the Great Lakes monitoring program operated over the past decade, Cairns (unpubl. data) has collected information on the prevalence of lip papillomas on white suckers in the western basin of Lake Ontario. Previous work suggested that the prevalence was increased in areas associated with heavy industrial contamination (Sonstegard, 1977). There have been several recent studies on the development of the papillomas (reviewed in Hayes et al., 1987a,b; Metcalfe et al., 1987).

The data set collected by Cairns represents the most complete set of data available for any single sucker population. The fish which were sampled can be separated into two groups based on the presence or absence of papillomas. Cairns provided us with a subsample of the data consisting of approximately six to nine suckers with and without papillomas from 12 sites in Lake Ontario. Data were analyzed by site for effects of tumor-presence on length, weight, fecundity and condition factor of female fish. There was a significant effect of tumor-presence, and fish with papillomas were significantly longer, heavier and had higher fecundity. This strongly suggested an association between tumor presence and age of the fish, and that this phenomenon deserves further attention.

Cairns has comprehensively compared the growth, mortality and reproductive performance of suckers with papillomas to non-tumored fish. Sucker collections for these studies were concentrated in the

Hamilton Harbour area and they found that papilloma frequency increased with age; 10% of fish were affected at 4 y, at 7 y, 20%, at 10-14 y, 40-70% and frequency may have declined after 17 y of age although the sample size was small (Cairns, unpubl. data).

No differences were detected in age and length relationships within the population, and no effects were detected on fecundity once the data had been standardized for age. There was also no detectable differences in the spawning behaviour or reproductive success of fish with papillomas. Fertilized eggs from fish with lesions were compared with non-lesioned fish and no differences could be detected with fertilization rate, hatching success or larval growth up to 71 d post-hatch.

Age-distribution data was collected during three successive spawning runs from fish collected at weirs, and no difference could be detected in the age distributions or mortality rates of suckers with papillomas.

Extensive data collections at other Lake Ontario sites has also failed to demonstrate a significant effect of papilloma presence on growth, mortality, reproductive success or larval survival. This may not be surprising, since the absence of a lesion may not mean that the fish has not been affected by stressors associated with induction of the papillomas. The lesions are known to alter in size, frequency and distribution over a 12 week holding period in lab water (Hayes et al., 1987a).

Although no significant impacts of the papillomas have been detected on the suckers, the PISCES framework correctly identified that the papillomas may be associated with the age of the fish and that this area represented an area warranting further study.

8.8 Conclusions

PISCES represents a mechanism for stock comparison and preliminary indications for focusing of a study. In each case, the conclusion offered by the PISCES framework was identical to the conclusion of the study group after the investment of intensive research efforts. The PISCES system has the potential to act as a tool for the preliminary diagnosis of ecosystem impact and to suggest areas for intensive follow-up study.

Chapter IX

GENERAL DISCUSSION

9.1 Summary of Results

The original objectives of the study were to

1. examine the sucker populations for effects of exposure to the metals
2. examine larval tolerance for impacts of metal exposure and for evidence of genetic selection
3. develop a cost-effective framework for the comparison of sucker populations.

Changes were detected in growth, reproduction and larval survival of the sucker populations exposed to metals. The changes can be separated into indirect effects of the metals on the food base of the suckers, and direct effects on the suckers.

9.1.1 Indirect effects

Changes in reproduction and growth were detected using an environmental health assessment approach. Female fish from the contaminated sites exhibited decreased size after maturation, decreased muscle lipid levels and decreased total serum lipid levels during the post-spawning period. The ability of the fish to attain sizes comparable

to control fish until maturation suggested that the fish were not capable of accumulating sufficient energy to meet the demands of both growth and reproduction. The lack of obvious effects on males is consistent with a hypothesis that food is limiting since females require more energy to sustain their reproductive efforts. The fish exhibited no differences from control groups in mean age, condition factor, catch per unit effort or sex ratio (Munkittrick and Dixon, 1988; Table 46). Females from the contaminated site did not differ in age at maturity, but exhibited increased spawning failure, decreased fecundity and decreased egg size.

At the contaminated sites, no effects were detected on gonadal development measured morphologically (GSI) or histologically (unpubl. data). Furthermore, there was no detectable impact on the fertilization performance of suckers at the contaminated site, either in the lab or *in situ* (Munkittrick and Dixon 1987). Eggs from contaminated sites, which were fertilized and incubated in clean water were smaller, and produced smaller larvae at hatch which developed and grew more slowly and showed a poorer conversion to feeding and a poorer survival (Munkittrick and Dixon, 1987). The decreased energetic commitment to reproduction is again suggestive of nutritional limitations.

The absence of a significant effect on the mean age of the fish restricts the response to a type IV or V pattern (Table 41), and the absence of a condition factor change strongly suggests that the fish

Table 46: Summary of the response of a white sucker populations to mixed metal mining wastes.

Site	Mean Age	Age Distribution	Growth Rate	Condition Factor	Age at Maturation	RLS	Fecundity	Egg Size	CPUE	Population Size
¹ MAN	6.55 ¹	shift to older ²	decreased ³ after maturity	2.10 ⁴	4.8	1.8	23000	1.70 ⁵	1.50 ⁶	see text
LOK (control)	6.34			2.17	4.1	2.2	31000	1.80	1.36	
Observed Change	0	+2	-	0	+	-	-	-	0	-
Pattern V	0	0	-	0	+	-	-	-	-	-

¹Ages for females not significantly different, values for males 7.30 and 5.90

²Shift in age distribution due to use of a single size gill net mesh (3.5") and the decreased growth rate at MAN; older LOK fish not collected

³No difference until after sexual maturation of females; males exhibited initial decrease after maturity and then an increase back to control levels

⁴Values for females not significantly different, values for males were 2.07 and 2.14 (significantly different)

⁵Mean diameter (mm)

⁶Comparison of fish hr⁻¹ using same net at both sites during fall of 85 and 86 from 1800 to 2400 hr

are exhibiting a type V response, characteristic of a habitat change or a niche shift. All of the responses of the females are consistent with this finding (Table 46). The conclusions of the PISCES approach are the same as for the overall analysis, and both suggest that food availability may be a problem.

Information available on the Manitouwadge chain suggests that benthic invertebrates are rare or absent from deeper areas (>5-7 m) of the contaminated lakes (German, 1971; Pugh and Maki, 1986). Suckers are known to be opportunistic feeders (Lalancette, 1977), and suckers at the contaminated site are known to be feeding on different organisms (unpubl. data). The metal contaminated lakes do not appear to be capable of supporting invertebrate fauna below 5-7 m of depth (German 1971; Pugh and Maki 1986) and several major food groups are absent or in low density in shallow areas at contaminated sites. The fauna of shallower areas around the contaminated lakes is dominated by chironomids and tubificids, and lacks, or has a marked decrease, in the abundance of mayflies, caddisflies, snails and clams found in control lakes in the chain (Pugh and Maki, 1986; Bunn, Munkittrick and Dixon, unpubl. data). Metal levels in the sediments of MAN average 102 mg kg^{-1} Cu and 1150 mg kg^{-1} Zn, and metal levels in the stomach contents of suckers exceed 1200 mg kg^{-1} of zinc (Miller et al., 1988). This data fits a type V response well and suggests that the majority of the effects on post-larval Manitouwadge suckers are indirect, as a result of alterations in the food base available for fish.

9.1.2 Direct effects

The decreased larval survival suggests some direct effects on the suckers. Eggs incubated in the contaminated streams exhibited a further decrease in size, increased deformities and did not perform as well when exposed to copper or zinc in the lab. The absence of an effect on mean age, size of fish (before maturation) or condition factor in light of the increased larval mortality does not fit a pattern consistent with a decrease in population size.

Van Loon and Beamish (1977) did not detect any effect of exposure to 90 ug l^{-1} Zn and 10 ug l^{-1} Cu on the growth, year-class strength or abundance of fish. However, some adverse effects were found in lakes with levels of zinc between 200 and 1000 ug l^{-1} (Van Loon and Beamish 1977). Removal of fish from a population as a result of contaminant impact should have the same effect on the population as removal by fishing. Such changes would be expected to induce density-dependent compensation. A relative increase in food resources would be expected to result in increased growth, earlier maturation and increased fecundity.

McFarlane and Franzin (1978) studied white suckers in a lake receiving atmospheric inputs of copper (13 ug l^{-1}), zinc (245 ug l^{-1}) and cadmium (0.6 ug l^{-1}), and found earlier maturation, decreased spawning success, decreased larval and egg survival, smaller egg size and decreased longevity in suckers (Table 43). Suckers in this study exhibited decreased growth and fecundity despite the presence of Cu

and Zn levels similar to the lakes examined by McFarlane and Franzin (1978). The absence of density-dependent compensation in Manitowadge suckers suggests that either mortalities associated with the metals are insufficient to induce compensatory mechanisms or that the metals have altered the environment so that the carrying capacity of the system has been reduced to a new level, consistent with a decreased population size. The carrying capacity of the contaminated lakes must decrease in association with the reduced food availability and the sucker population may have reached a new equilibrium within a system whose capacity for raising suckers is much lower than existed pre-contamination. The absence of food in deeper water within the contaminated lakes may account for a relative increase in the prevalence of suckers in nearshore areas, and the apparent equality of CPUE between sites.

It seems likely that most of the changes evident in these populations of suckers are a result of an indirect effect of the metals on the food items of the fish in the contaminated lakes. The apparent differences in the response of MAN suckers compared to those studies by McFarlane and Franzin (1978; Table 43) may relate to differences in exposure. Increases in waterborne metal levels arising from atmospheric deposition would occur slowly, and without the relatively high sediment metal burdens associated with a direct input from mining wastes. Although levels of Cu and Zn in both lake systems are comparable at the present time, waterborne metal levels at the MAN

site are approximately half of those reported by German (1971). Furthermore, sediment levels have declined from more than $700 \text{ mg kg}^{-1} \text{ Cu}$ and $4400 \text{ mg kg}^{-1} \text{ Zn}$ (German, 1971) to present levels of approximately 100 and 1150 mg kg^{-1} respectively. Presumably the contamination of lakes by atmospheric deposition (McFarlane and Franzin 1978) would not involve levels of sediment contamination comparable with past records at the Manitouwadge site. Levels in the sediments of the Manitoba study lakes may take some time to reach those levels present in the Manitouwadge system two decades ago. Consequently, invertebrate populations may have been spared the eradication associated with sediment contamination in the Manitouwadge lakes.

9.2 Conclusions

The decreased energetic commitment of females to reproduction, decreased growth after sexual maturation and larval changes are consistent with the findings of decreased food availability associated with increased sediment loading of metals. Direct effects appear to be restricted to effects on egg development and an increased deformity rate of larvae.

The origin of the maternal factor is still unknown, but the benefits seem well-suited to the environment. The increase in tolerance lasts until the completion of yolk absorption, which occurs some time after swim-up of the larvae. At the time of swim-up, the larvae rise to the surface and are carried downstream to the lake, where metal

levels are substantially lower than the stream. The duration of increased tolerance coincides with the period of time when the larvae are most susceptible and are exposed to the highest levels of water-borne metals. The benefits of increased tolerance associated with a maternal yolk factor may be partially offset by incubation of eggs in the streams flowing out of tailings ponds.

The PISCES approach correctly identified the population response, and correctly suggested the area for follow-up study. The fact that PISCES detected changes in wild fish exposed to several classes of contaminants, at contamination levels close to the water quality criteria is encouraging. When differences were not clearly definable, inconsistencies clearly suggested areas for follow-up studies with intensified effort. Also encouraging was the fact that PISCES detected changes related to indirect effects of metals on the food base of suckers. The detection of such indirect effects emphasizes the utility of a surveillance framework such as this one.

This framework is not restricted to suckers, and undoubtedly could be easily applied to percids (Colby and Nepszy, 1981; Colby, 1984), salmonids (Ryder and Edwards, 1985), and coregonids (George, 1977; Healey, 1978). However, extreme caution must be used to compare stocks with similar fishing intensities. Experimentally, this framework offers a holistic approach to field surveillance, encouraging analysis from the perspective of the whole animal. If changes exist among the whole organisms, then further, more expensive work

can be initiated to examine speciation states, body burdens and other contaminant phenomena associated with the alterations.

9.3 Relative Value of PISCES and EHA

The best understanding of the interactions of unknown chemicals in the environment would be through a progression of studies from the ecosystem level to individual, biochemical responses. Such a study requires a massive investment of both time and financial resources, and is not feasible for use in a general surveillance program. The environmental health assessment was time-consuming, expensive and was only successful at suggesting areas for follow-up study. The approximate cost of the full field study was in excess of \$100,000 over the three year period. The collection of 30 female fish from a spawning run would cost a maximum of \$1000- \$2000, depending on site access. Despite the marked differences in cost, for all studies reviewed the PISCES approach correctly identified the areas requiring follow-up study. The use of such a program could allow the field surveillance of 50 to 100 areas for the price of a single, comprehensive assessment. A standardized approach is needed so that an increase in field observations will increase the availability of information for interpreting other studies. An evaluation framework like PISCES would be capable of eliminating areas without obvious impact from further costly studies, and could rapidly and cheaply define areas where research resources could be invested wisely.

9.4 Limitations

Ideally, any testing framework should be inexpensive, require a minimum of expertise for sample collection or storage, and have few equipment requirements in the field. Predictability can not be based on the direct observation of environmental impact, and must incorporate observations on similar systems and an understanding of interactions occurring under the presence of various stressors and abiotic modifying factors (Cairns, 1981).

PISCES has been shown to be applicable, easy to do, of relevance to aquatic ecosystems, and is scientifically defensible. The general limitations of the PISCES framework are :

1. there may be no dose-response effects due to compensation responses at low contaminant exposures. Density-dependent compensation mechanisms may not be obvious, and may be altered by influences independent of contaminant exposure. The identification of cause and effect relationships can be difficult or impossible.
2. alterations which do not affect growth, survivorship or reproduction will not be detected. Influences which do not affect survival, such as behavioural changes, may be difficult to identify, and changes related to age-specific mortality can be obscured by sampling inadequacies. Furthermore, the absence of obvious population effects does not guarantee that the ecosystem will be capable of tolerating subsequent stressors.

3. the degree of response to negative feedback varies with the energy and nutrient availability within a system. The response can be species-specific, with the response being dependent upon the species' potential for elevation of fecundity in response to environmental or exploitative pressures (Colby and Nepszy, 1981; Neuhold, 1987).
4. the framework is reactive and retrogressive, changes can only be detected after significant impact has occurred on the populations and then evidence is only circumstantial. The entire framework is based on the selection of a comparable reference site. Since there can never be a perfect control, comparisons are made between similar stocks (stressed versus unstressed) in similar environments with the same type of sampling procedures. Attempts to simplify relationships must be treated with caution, and must be interpreted as evidence to provide future direction for assessment and not as final conclusions. The disadvantages of poor specificity and obscure dose-response relationships (Hodson, 1987b) can be satisfied by follow-up testing involving the use of appropriate biochemical tests (Hodson, 1986, 1987a,b) when differences are apparent.
5. the impacts of contaminants on different ages or sexes of fish have not been treated separately, although this would be easy to do.

6. the framework does not require an evaluation of the reproductive success or larval survival of the sucker populations. Such information was essential for the complete understanding of the effects of mine waste and atmospheric deposition. An assessment of larval survival would be valuable in any evaluation to detect impacts on early mortality.

9.5 Future Needs

Cairns (1981) estimates that biological assessment and monitoring of pollutional effects will develop through four phases: awareness, observation, prediction and management. The awareness and observational phases have obviously been underway for several decades, and there has not been a stepwise shift from one phase to the next. Predictive attempts have been in the literature for 10-15 years and the first true management reports are very recent (Pearce, 1984; Chapman et al., 1985). A restriction of the initiation of the management phase has been the development of managerial tools and the necessity of evaluation on a case by case basis (Cairns, 1981). The limited predictive capabilities and deficiencies of past impact statements were generally associated with the gathering of information or its analysis, using a diversity of methods or species, the absence of data on transformation and partitioning of the contaminants, or the inadequate coupling of such data to the biological data (Cairns, 1981). Such frameworks must be inexpensive, and require evaluation

in different geographical areas and ecological settings, and with different stressors and contaminant combinations (Pearce, 1984). The development of managerial techniques will not be rapid or inexpensive, but it is hoped that this paper may initiate some thought and discussion.

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Appendix A

ABBREVIATIONS

AGN	Agonzon Lake
ALPB	alkali-labile protein-bound phosphorus, an indirect indicator of serum vitellogenin levels
CCREM	Canadian Council of Resource and Environment Ministers
CPUE	catch per unit effort
DTU	daily temperature units, calculated as the number of days multiplied by the temperature (°C)
EPA	Environmental Protection Agency
FAAS	flame atomic absorption spectrophotometry
GSI	gonadosomatic index
HDL	high-density lipoprotein cholesterol
HPI	hypothalamo-pituitary-interrenal axis
k	condition factor, calculated as $\text{weight}(100)/\text{length}^3$.
KAG	Kaginu Lake
LC50	the concentration of a toxicant required to kill 50% of a population
LL	MAN eggs fertilized in water from LOK and incubated at the LOK site
LM	MAN eggs fertilized in LOK water and incubated at the MAN site
LMN	Little Manitouwadge Lake

LMS	Little Mose Lake
LOK	Loken Lake, the control site
LOnt	Lake Ontario collections of eggs
LSI	liversomatic index, calculated as $\text{liver weight}(100)/\text{body weight}$
LT50	the time required for 50% of a population to die
MAN	Manitouwadge Lake
ML	MAN eggs fertilized in MAN water and incubated at LOK
MM	MAN eggs fertilized in MAN water and incubated at MAN
MOS	Mose Lake
MS222	tricaine methanesulphonate, an anaesthetic
MT	metallothionein
NBS	National Bureau of Standards
PISCES	Population Indicators of Sublethal Contaminant Effects on Suckers
POST	Postspawning period
PRES	Prespawning period
RECR	Recrudescent period, the period of gonadal growth
RLS	Reproductive life span
SE	standard error
SPAW	Spawning period
STR	Straight Lake
WOW	Wowun Lake
Z _{ag.}	age to maturation

Appendix B

FISH SAMPLES

This appendix lists all of the suckers collected during this study by site, sex and season of collection.

Legend:

Site 1 MAN, 2 LMN, 3 MOS, 5 WOW, 6 LOK

Blood B Blood collected, NB No blood sample

G WT gonad weight

SITE	SEASON	NO	BLOOD		LENGTH	G WT		HSI
YR	SEX		TIME	AGE	WEIGHT	GS		
1	85	POST	M	107	PM NB	9	36.0 826 18.0	2.2
1	85	POST	M	108	PM NB	4	30.0 604 22	3.6
1	85	POST	M	109	PM NB	5	30.5 491 10.0	2.0
1	85	POST	M	111	PM NB	.	30.5 606 10	1.7
1	85	POST	M	112	PM NB	9	35.0 1000 19	1.9
1	85	POST	M	117	PM B	8	32.0 726 10	1.4
1	85	POST	M	122	PM NB	5	31.5 565 17	3.0
1	85	POST	M	123	PM NB	6	29.0 540 10	1.8
1	85	RECR	M	131	PM NB	7	32 750 40	5.3
1	85	RECR	M	134	PM B	8	33.0 740 42.0	5.7 0.5
1	85	RECR	M	137	PM B	4	32.5 775 48.0	6.2
1	85	RECR	M	139	PM B	7	33.5 750 68.5	9.1
1	85	RECR	M	141	PM B	5	30.5 650 36	5.5
1	85	RECR	M	149	AM B	4	29.5 555 39.5	7.1 0.9
1	85	RECR	M	151	PM B	10	33.5 775 46	5.9 1.0
1	85	RECR	M	155	AM B	5	32.0 695 33	4.7 0.8
1	85	POST	F	103	PM NB	10	32.0 737 10	1.4
1	85	POST	F	104	PM NB	7	31.0 750 79.2	10.6
1	85	POST	F	105	PM NB	4	30.5 713 98.0	13.7
1	85	POST	F	106	PM NB	7	30.5 706 88.0	12.5
1	85	POST	F	113	PM NB	4	32.0 636 76.0	11.9
1	85	POST	F	114	PM NB	7	32.0 720 10	1.4
1	85	POST	F	115	PM B	4	29.5 581 10	1.8
1	85	POST	F	116	PM B	5	30.5 691 10	1.4
1	85	POST	F	119	PM NB	4	32.0 612 10	1.6
1	85	POST	F	120	PM NB	6	31.0 670 10	1.6
1	85	POST	F	121	PM B	4	33.5 820 10	1.2

SITE	SEASON	NO	BLOOD	LENGTH	G WT	HSI
YR	SEX	TIME	AGE	WEIGHT	GSI	
1 85	POST	F	124 PM B 4	31.0 780	80.0 10.3	.
1 85	POST	F	125 PM NB 5	31.0 670	92.0 13.7	.
1 85	POST	F	126 PM NB 6	37.0 1080	10 0.9	.
1 85	RECR	F	128 PM B 4	33.5 950	38.0 4.0	.
1 85	RECR	F	129 PM B 4	34.0 860	40.5 4.7	.
1 85	RECR	F	130 PM NB 4	32 795	33 4.2	.
1 85	RECR	F	132 PM NB 5	34 700	26 3.7	.
1 85	RECR	F	133 PM B 5	33 730	27.5 3.8	0.7
1 85	RECR	F	135 PM B 4	33 740	30.5 4.1	1.1
1 85	RECR	F	136 PM B 3	36.5 945	31 3.3	1.3
1 85	RECR	F	138 PM B 8	36.5 940	48 5.1	.
1 85	RECR	F	140 PM B 8	37.5 930	42 4.5	.
1 85	RECR	F	142 PM B 7	35 1035	45.5 4.4	.
1 85	RECR	F	143 PM NB 6	33 730	30 4.1	.
1 85	RECR	F	144 PM NB 6	34 840	38 4.5	.
1 85	RECR	F	145 PM NB 5	33 750	29 3.9	.
1 85	RECR	F	146 AM B 5	32.5 765	33 4.3	1.1
1 85	RECR	F	147 AM B 5	32.5 695	27 3.9	1.1
1 85	RECR	F	148 AM B 8	33.5 700	31 4.4	1.1
1 85	RECR	F	150 AM B 7	35 830	44.5 5.4	1.6
1 85	RECR	F	152 PM NB 10	43 1505	90 6.0	1.4
1 85	RECR	F	153 PM NB 6	33 785	23 2.9	0.9
1 85	RECR	F	156 AM NB 6	36 1130	49 4.3	0.9
1 85	RECR	F	157 AM NB 6	32 665	26 3.9	1.3
1 86	PRES	M	701 PM B 8	36 908	49.9 5.5	.
1 86	PRES	M	702 PM B 6	32.5 585	26.4 4.5	.
1 86	PRES	M	703 PM B 7	30 555	19.3 3.5	.
1 86	PRES	M	704 PM B 7	31 585	31.6 5.4	.
1 86	PRES	M	705 PM B 9	33 700	30.8 5.4	.
1 86	PRES	F	706 PM B 9	34.5 755	89.9 11.9	.
1 86	PRES	F	707 PM NB 10	40 1170	169 14.5	.
1 86	PRES	F	708 PM B .	33 705	75.6 10.7	.
1 86	PRES	F	709 PM B 6	33 785	109 13.9	.
1 86	PRES	F	710 PM B 6	33.5 710	74.2 10.5	.
1 86	PRES	F	711 PM B 8	34 805	75.6 11.9	.
1 86	PRES	M	712 PM B 9	34.5 740	32.9 4.4	1.62
1 86	PRES	M	713 PM NB 7	33 675	22.8 3.4	1.56
1 86	PRES	M	714 PM NB 5	29.5 510	29.2 5.7	1.22
1 86	PRES	F	715 PM NB 5	32 655	79.7 12.2	1.77
1 86	PRES	F	716 PM NB 8	32.5 705	94.9 13.5	1.97
1 86	PRES	M	717 PM NB 7	34 660	32.1 4.9	1.68
1 86	PRES	M	718 PM NB 9	34.5 660	33.2 5.0	1.21
1 86	PRES	M	719 PM NB 7	34 705	36.9 5.2	1.72
1 86	PRES	F	720 PM NB 7	34.5 660	81.3 12.3	1.82
1 86	PRES	F	721 AM B 6	35 775	105 13.6	1.34
1 86	PRES	M	722 AM B 4	29 455	17.8 3.9	1.05
1 86	PRES	M	723 AM B 9	31.5 685	28.4 4.2	1.27

SITE	SEASON	NO	BLOOD	LENGTH	G WT	HSI
YR	SEX	TIME	AGE	WEIGHT	GSI	
1 86	PRES	F	724	AM	B 7	34 770 71.8 9.3 1.36
1 86	PRES	F	725	AM	B 8	33.5 830 93.6 11.3 1.53
1 86	PRES	M	726	AM	B 9	36.5 1030 45.1 4.4 2.05
1 86	PRES	F	727	AM	B 8	32.5 780 92.1 11.8 2.05
1 86	PRES	M	728	AM	B 8	34.0 795 33.2 4.2 1.24
1 86	PRES	F	729	AM	B 7	30.5 680 71.1 10.5 1.76
1 86	PRES	F	730	AM	B 6	32.0 740 61.2 8.3 1.51
1 86	PRES	M	731	AM	B 8	33.5 745 32.7 4.4 1.93
1 86	PRES	M	732	AM	B 7	33.0 715 25.7 3.6 1.34
1 86	PRES	M	733	AM	B 8	34.0 850 43.8 5.2 1.55
1 86	PRES	F	734	AM	NB 6	32.0 670 63.1 9.4 .
1 86	PRES	M	735	AM	NB 6	31.0 650 29.2 4.5 .
1 86	PRES	M	736	AM	NB 10	37.0 1035 . . .
1 86	PRES	F	737	AM	NB 10	36.0 975 124 12.6 .
1 86	PRES	M	738	AM	NB 6	30.5 615 25.3 4.1 .
1 86	PRES	F	739	AM	NB 8	32.0 730 84.3 11.6 .
1 86	PRES	F	740	AM	NB 6	34.5 880 98.8 11.2 .
1 86	PRES	M	741	AM	NB 5	30.0 555 5.6 1.0 .
1 86	PRES	M	742	AM	NB 10	34.5 865 42.6 4.9 .
1 86	PRES	F	743	AM	NB 10	37.0 980 157 16 .
1 86	SPAW	F	744	PM	B
1 86	SPAW	F	745	PM	B
1 86	SPAW	F	746	PM	B
1 86	SPAW	F	747	PM	B
1 86	SPAW	F	748	PM	B
1 86	SPAW	F	749	PM	B
1 86	SPAW	M	750	PM	B
1 86	SPAW	M	751	PM	B
1 86	SPAW	M	752	PM	B
1 86	SPAW	M	753	PM	B
1 86	SPAW	M	754	PM	B
1 86	SPAW	M	755	PM	B
1 86	POST	F	757	AM	B 7	31.5 730 85.0 11.6 1.29
1 86	POST	M	758	AM	B 6	32 705 13.3 1.9 1.21
1 86	POST	M	759	AM	B 7	34 680 15.8 2.3 1.99
1 86	POST	M	760	AM	B 4	29.5 595 28.5 4.8 1.51
1 86	POST	M	761	AM	B 5	30.5 535 11.5 2.2 1.31
1 86	POST	F	763	PM	B 6	34.5 720 . 3.0 1.74
1 86	POST	F	765	PM	B 6	36 865 . 3.0 1.68
1 86	POST	F	767	PM	B 6	31 560 8.6 1.5 1.70
1 86	POST	M	768	PM	B 6	31 555 16.1 2.9 1.86
1 86	POST	F	769	PM	B 5	33 635 13.8 2.2 2
1 86	POST	F	770	PM	B 6	32 625 14.7 2.4 2.18
1 86	POST	M	771	PM	B 7	32.5 725 12.1 1.7 1.45
1 86	POST	F	772	PM	B 10	38.5 1030 19.4 1.9 1.41
1 86	POST	F	775	PM	B 10	37 1075 22.7 2.1 .

SITE	SEASON	NO	BLOOD		LENGTH	G WT		HSI
YR	SEX	TIME	AGE	WEIGHT	GSI			
1	86	RECR	F	301	PM NB	5	30.5 575 19 3.3	1.56
1	86	RECR	F	302	PM NB	4	29.5 545 7 1.3	1.10
1	86	RECR	F	303	PM NB	8	36 905 60 6.6	1.55
1	86	RECR	M	308	AM NB	4	34 760 48 6.3	1.18
1	86	RECR	M	309	AM NB	6	32 645 34 5.3	1.24
1	86	RECR	F	310	AM NB	5	30.5 585 40 6.8	1.54
1	86	RECR	F	314	AM NB	6	35.5 865 68 7.9	1.85
1	86	RECR	M	315	AM NB	5	34 675 40 5.9	1.33
1	86	RECR	F	316	AM NB	5	33 695 35 5.0	1.73
1	86	RECR	F	317	AM NB	5	30.5 575 39 6.8	1.74
1	86	RECR	F	318	AM NB	6	34.5 880 64 7.3	1.36
1	86	RECR	M	321	AM NB	7	35 795 53 6.7	1.13
1	86	RECR	M	322	AM NB	4	33 675 43 6.4	1.33
1	86	RECR	M	323	AM NB	5	31.5 675 52 7.7	1.33
1	87	POST	M	MN1	AM NB	.	34.0
1	87	POST	F	MN2	AM NB	.	33.5
1	87	POST	M	MN3	AM B	.	30.0
1	87	POST	M	MN4	AM B	.	31.0
1	87	POST	F	MN5	AM B	.	32.5
1	87	POST	F	MN6	PM B	.	30.0
1	87	POST	F	MN7	PM B	.	32.0
1	87	POST	M	MN8	PM NB	.	30.5
1	87	POST	F	MN9	PM NB	.	34.0
1	87	POST	F	MN10	PM B	.	29.5
1	87	POST	F	MN11	PM B	.	35.5
1	87	POST	F	MN12	PM B	.	32.5
2	85	POST	M	202	PM B	6	29.5 580 10 1.8	.
2	85	POST	M	203	PM B	.	33.5 820 10 1.2	.
2	85	POST	M	204	PM B	8	33.5 720 10 1.4	.
2	85	POST	M	206	PM B	9	34.5 1005 10.0 1.0	.
2	85	POST	M	208	PM B	10	34.5 800 10 1.3	.
2	85	POST	M	209	PM NB	10	32.0 1060 10 0.9	.
2	85	POST	M	210	PM NB	10	34.5 860 10 1.2	.
2	85	POST	M	213	PM B	8	31.5 610 10 1.6	.
2	85	POST	M	217	PM NB	7	32.5 790 10 1.3	.
2	85	POST	M	218	PM NB	8	33.5 780 10 1.3	.
2	85	POST	M	219	PM NB	6	29.0 580 10 1.8	.
2	85	POST	M	220	PM NB	9	35.0 900 10 1.1	.
2	85	POST	M	221	PM NB	6	32.0 720 10 1.4	.
2	85	POST	M	222	PM NB	10	34.0 680 10 1.5	.
2	85	POST	M	223	PM NB	7	30.0 620 10 1.6	.
2	85	POST	M	225	PM NB	10	30.5 660 10 1.5	.
2	85	POST	M	226	PM NB	8	33.5 720 10 1.4	.
2	85	RECR	M	232	PM NB	5	31.0 650 42.0 6.5	.
2	85	RECR	M	234	PM B	9	35.5 970 84 8.7	.
2	85	RECR	M	235	PM NB	5	29.0 559 32.0 5.7	.
2	85	RECR	M	236	PM B	8	34.5 1105 81 7.3	.

SITE	SEASON	NO	BLOOD		LENGTH	G WT		HSI
YR	SEX		TIME	AGE	WEIGHT	GSI		
2	85	RECR	M	237	PM B	9	35.0 980 59	6
2	85	RECR	M	238	PM NB	5	31.0 770 48	6.2
2	85	RECR	M	239	PM NB	6	36.0 1055 4	0.4
2	85	RECR	M	241	PM B	5	33.5 895 41	4.6
2	85	RECR	M	242	AM B	9	33.5 810 52	5.2
2	85	RECR	M	243	AM B	7	32.5 585	7.5
2	85	RECR	M	244	AM B	5	31.5 630	7
2	85	RECR	M	245	AM B	10	36.0 1065 75	7
2	85	RECR	M	246	AM B	6	32.0 685 41.5	6.1
2	85	RECR	M	247	AM B	6	32.5 765 39	5.1
2	85	RECR	M	249	PM B	5	34.5 875 66.5	7.7
2	85	RECR	M	250	PM B	10	34.5 865 72.5	8.4
2	85	RECR	M	251	PM NB	10	34 865 54	6.2
2	85	POST	F	201	PM NB	.	29.5 650 10.0	1.5
2	85	POST	F	205	PM B	8	32.0 720 10	1.4
2	85	POST	F	207	PM B	9	33.5 720 10	1.4
2	85	POST	F	211	PM B	5	32.0 720 10	1.4
2	85	POST	F	212	PM B	6	33.5 760 10	1.3
2	85	POST	F	214	PM B	5	38.0 1040 10	1.0
2	85	POST	F	215	PM B	8	32.5 760 10	1.3
2	85	POST	F	216	PM B	5	31.5 750 10	1.3
2	85	POST	F	224	PM NB	8	31.0 710 10	1.4
2	85	POST	F	227	PM NB	6	31.0 620 10	1.6
2	85	POST	F	228	PM NB	.	33.0 780 10	1.3
2	85	POST	F	229	PM NB	.	33.0 710 10	1.4
2	85	RECR	F	230	PM B	4	35.5 895 38.0	4.2
2	85	RECR	F	231	PM B	5	29 590 4.8	0.8
2	85	RECR	F	233	PM B	9	32.5 685 26.0	3.8
2	85	RECR	F	240	PM B	5	33 915 40	4.4
2	85	RECR	F	248	AM B	5	34 750 33	4.4
2	85	RECR	F	252	PM B	5	34.5 785 39.5	5
2	85	RECR	F	253	PM B	5	34.5 950 37	3.9
2	85	RECR	F	254	PM NB	6	37.0 1115 56	5
2	85	RECR	F	255	PM NB	.	33.5 805 36	4.5
2	85	RECR	F	256	AM B	5	33 770 31.5	4.1
2	85	RECR	F	257	AM B	6	34 875 42	4.8
2	85	RECR	F	258	AM B	6	35 880 43	4.9
2	85	RECR	F	259	AM B	5	31 510 31	6.1
2	86	PRES	M	801	AM NB	6	31 580 24	4.3
2	86	PRES	F	802	AM NB	6	32.5 835 84	10.1
2	86	PRES	M	803	AM NB	7	31.5 625 22.5	3.6
2	86	PRES	F	804	AM NB	8	34 920 120	13.0
2	86	PRES	M	805	AM NB	10	36 905 44.9	5.0
2	86	PRES	F	806	AM NB	7	33.5 900 104	11.5
2	86	PRES	M	807	AM NB	6	31 555 22.5	4.1
2	86	PRES	M	808	AM NB	5	31 680 26.9	4.0
2	86	PRES	F	809	AM NB	7	35 890 120	13.4

SITE	SEASON	NO	BLOOD	LENGTH	G WT	HSI
YR	SEX	TIME	AGE	WEIGHT	GSI	
2 86	PRES	F	810	AM NB 7	32.5 825 99.4 11.6	1.89
2 86	PRES	M	811	AM NB 6	29 605 27.7 4.6	.
2 86	PRES	M	812	AM NB 7	32.5 660 30.6 4.6	.
2 86	PRES	M	813	AM NB 7	33 620 28.3 4.6	1.45
2 86	PRES	M	814	PM B 8	33.5 685 35.5 5.2	1.43
2 86	PRES	M	815	PM B 6	33 725 31.7 4.4	1.82
2 86	PRES	M	816	PM B 10	35 940 49 5.2	1.91
2 86	PRES	M	817	PM NB 6	29.5 545 22 4.0	1.24
2 86	PRES	M	818	PM B 6	32 660 23 3.5	1.41
2 86	PRES	M	819	AM B 9	32 680 30.7 4.5	1.26
2 86	PRES	M	820	AM B 7	32.5 695 25.3 3.4	1.57
2 86	PRES	M	821	AM NB 10	30.5 600 22.3 3.7	1.61
2 86	PRES	F	822	AM B 7	33 685 68.8 10.0	1.52
2 86	PRES	F	823	AM B 8	34 735 76.1 10.4	1.96
2 86	PRES	M	824	AM B 7	32.5 610 25.5 4.2	1.48
2 86	PRES	F	825	AM B 10	41 1475 187 12.7	1.65
2 86	PRES	F	826	AM B 9	32.5 675 78.5 11.6	1.78
2 86	PRES	M	827	AM B 6	32 700 26.9 3.8	1.10
2 86	PRES	F	828	AM B 7	34.5 830 94.7 11.4	1.93
2 86	PRES	F	829	AM B 7	31.5 785 85.6 10.9	1.66
2 86	PRES	F	830	AM NB 6	31 . 3.9 .	.
2 86	PRES	M	831	AM B 8	30 670 29.5 4.4	1.28
2 86	PRES	F	832	PM NB 7	32.5 640 53.9 8.4	.
2 86	PRES	F	833	PM NB 8	35.5 945 104 10.0	.
2 86	PRES	F	834	PM NB 7	31.5 650 79.8 12.3	.
2 86	PRES	F	835	PM NB 6	35 870 114 13.2	.
2 86	PRES	F	836	PM NB 7	33 740 70.9 9.6	.
2 86	PRES	F	837	PM NB 7	35 860 105 12.2	.
2 86	PRES	F	838	PM NB 8	32.5 780 71.8 9.2	.
2 86	PRES	F	839	PM NB 8	33 835 84.3 10.1	.
2 86	PRES	F	840	PM NB 6	32 740 74.3 10.0	.
2 86	PRES	F	841	PM NB 7	35.5 950 90.8 9.6	.
2 86	PRES	M	842	PM B 6	31.5 680 31.2 4.6	2.32
2 86	PRES	F	843	PM B 7	30.5 580 10.0 2	.
2 86	PRES	F	844	PM B 6	32.5 707 94 13.3	1.53
2 86	PRES	F	845	PM B 6	33 730 82.1 11.3	1.42
2 86	PRES	F	846	PM B 7	33 856 99.2 11.6	1.67
2 86	SPAW	M	847	PM B
2 86	SPAW	M	848	PM B
2 86	SPAW	M	849	PM B
2 86	SPAW	M	850	PM B
2 86	SPAW	M	851	PM B
2 86	SPAW	M	852	PM B
2 86	SPAW	F	853	PM B
2 86	SPAW	F	854	PM B
2 86	SPAW	F	855	PM B
2 86	SPAW	F	856	PM B

SITE	SEASON	NO	BLOOD		LENGTH	G WT		HSI				
YR	SEX		TIME	AGE		WEIGHT	GS I					
2	86	SPAW	F	857	PM	B	.	.				
2	86	POST	M	858	AM	B	9	35.5				
2	86	POST	M	859	AM	B	6	33				
2	86	POST	M	860	AM	NB	7	32.5				
2	86	POST	M	861	AM	B	9	39				
2	86	POST	F	864	AM	B	10	39.5				
2	86	POST	F	865	AM	B	9	34.5				
2	86	POST	M	866	AM	B	7	31				
2	86	POST	M	867	AM	B	7	31.5				
2	86	POST	F	868	AM	B	6	34				
2	86	POST	F	869	PM	B	7	32				
2	86	POST	M	871	PM	B	6	31.5				
2	86	POST	M	872	PM	B	10	31.5				
2	86	POST	F	873	PM	B	7	31				
2	86	POST	M	874	PM	B	5	31				
2	86	POST	F	875	PM	B	5	32				
2	86	POST	M	876	PM	B	6	32.5				
2	86	POST	F	877	PM	B	7	34				
2	86	POST	F	878	PM	B	10	42				
2	86	POST	F	879	PM	B	6	33				
2	86	POST	F	880	PM	NB	6	32				
2	86	POST	F	881	PM	NB	6	33.5				
2	86	POST	F	882	PM	NB	5	31.5				
2	86	POST	F	883	PM	NB	6	34				
2	86	POST	M	884	PM	B	9	32.5				
2	86	POST	M	885	PM	B	6	30				
3	86	RECR	F	1	AM	NB	9	33				
3	86	RECR	F	2	AM	NB	7	34.5				
3	86	RECR	F	3	AM	NB	10	37				
3	86	RECR	M	4	AM	NB	6	29				
3	86	RECR	M	5	AM	NB	8	34				
3	86	RECR	M	6	AM	NB	10	34.5				
3	86	RECR	M	7	AM	NB	8	32				
3	86	RECR	M	8	AM	NB	7	31.5				
3	86	RECR	M	9	AM	NB	7	32				
3	86	RECR	F	10	AM	NB	7	34.5				
3	86	RECR	F	11	AM	NB	10	37.5				
3	86	RECR	F	12	AM	NB	10	37				
3	86	RECR	M	13	AM	NB	6	33				
3	86	RECR	M	14	AM	NB	10	36				
3	86	RECR	F	15	AM	NB	7	40				
3	86	RECR	F	16	AM	NB	6	33.5				
3	86	RECR	F	17	AM	NB	10	35				
3	86	RECR	M	18	AM	NB	9	35				
3	86	RECR	M	19	AM	NB	6	33				
3	86	RECR	F	20	AM	NB	9	35				

SITE	SEASON	NO	BLOOD	LENGTH	G WT	HSI
YR	SEX	TIME	AGE	WEIGHT	GSI	
3	86	RECR	F	21	AM NB 5	34.5 750 . . .
3	86	RECR	F	22	AM NB 10	33.5 745 . . .
3	86	RECR	M	23	AM NB 10	36.5 990 . . .
3	86	RECR	F	24	AM NB 10	33 745 . . .
3	86	RECR	F	25	AM NB 10	34.5 835 . . .
3	86	RECR	M	26	AM NB 6	36.5 850 . . .
3	86	RECR	F	27	AM NB 6	35 855 . . .
3	86	RECR	F	28	AM NB 6	36.5 830 . . .
3	86	RECR	F	29	AM NB 6	33 650 . . .
3	86	RECR	F	30	AM NB 8	39.5 1265 . . .
3	86	RECR	F	31	AM NB 5	33 720 . . .
3	86	RECR	F	32	AM NB 5	32.5 715 . . .
3	86	RECR	F	33	AM NB 7	31 640 . . .
3	86	RECR	F	34	AM NB 7	33.5 730 . . .
3	86	RECR	F	35	AM NB 9	32 700 . . .
3	86	RECR	F	36	AM NB 9	38 1100 . . .
5	85	POST	M	501
5	85	POST	F	502
5	87	POST	F	503	PM NB .	31.0 . . .
5	87	POST	F	504	PM NB .	30.5 . . .
5	87	POST	F	505	PM NB .	29.5 . . .
5	87	POST	M	506	PM NB .	30.0 . . .
5	87	POST	F	507	PM NB .	31.5 . . .
5	87	POST	F	508	PM NB .	32.0 . . .
5	87	POST	F	509	PM NB .	30.0 . . .
5	87	POST	F	510	PM NB .	27.0 . . .
5	87	POST	M	511	PM B .	29.5 . . .
5	87	POST	F	512	PM B .	31.5 . . .
5	87	POST	M	513	PM B .	28.5 . . .
5	87	POST	F	514	PM B .	34.0 . . .
5	87	POST	F	515	PM B .	35.0 . . .
6	85	POST	M	603	PM B 10	34.5 850 10.0 1.2 .
6	85	POST	M	609	PM NB 7	33.5 810 10 1.2 .
6	85	POST	M	615	PM NB 7	37.0 1060 10 0.9 .
6	85	POST	M	617	PM B 6	34.0 835 10 1.2 .
6	85	POST	M	619	PM B 6	33.5 940 10 1.0 .
6	85	POST	M	622	PM NB 4	29.0 560 10 1.8 .
6	85	POST	M	624	PM B 9	34.0 930 10 1.0 .
6	85	POST	M	625	PM B .	37.0 1120 10 0.9 .
6	85	RECR	M	630	PM NB 5	33.0 765 39.0 5.1 0.8
6	85	RECR	M	636	PM B 7	34.5 930 58 6.2 .
6	85	RECR	M	638	PM NB 4	31 660 38.5 5.8 0.8
6	85	RECR	M	642	PM B 4	29.5 535 32.5 6.1 0.7
6	85	RECR	M	644	PM B 3	31.5 655 42.5 6.5 0.8
6	85	RECR	M	648	AM B 4	32 750 51 6.8 0.8
6	85	RECR	M	653	AM B 5	35 865 71 8.2 0.9
6	85	RECR	M	654	AM B 4	32 635 45 7.1 0.9

SITE	SEASON	NO	BLOOD	LENGTH	G WT	HSI
YR	SEX		TIME	AGE	WEIGHT	GSI
6 85	POST	F 601	PM B 7	36.5	1177 10.0	0.8 .
6 85	POST	F 602	PM B 8	37.0	1215 8	0.7 .
6 85	POST	F 604	PM B 9	42.0	1310 10	0.8 .
6 85	POST	F 605	PM B 6	35.0	1030 10	1.0 .
6 85	POST	F 606	PM B 5	32.5	950 91.0	9.6 .
6 85	POST	F 607	PM B 5	32.5	770 5	0.6 .
6 85	POST	F 608	PM B 7	31.0	715 10	1.4 .
6 85	POST	F 610	PM NB 5	32.0	925 99.0	10.7 .
6 85	POST	F 611	PM NB 5	31.5	780 10	1.3 .
6 85	POST	F 612	PM NB 7	36.0	1150 10	0.8 .
6 85	POST	F 613	PM NB 7	36.0	1140 10	0.8 .
6 85	POST	F 614	PM NB 4	33.0	850 10	1.2 .
6 85	POST	F 616	PM NB 6	36.0	1000 10	1.0 .
6 85	POST	F 618	PM B 7	35.0	860 10	1.2 .
6 85	POST	F 620	PM B 6	36.5	1139 10	0.8 .
6 85	POST	F 621	PM NB 6	31.0	675 10	1.5 .
6 85	POST	F 623	PM NB 10	41.0	1515 10	0.7 .
6 85	RECR	F 626	PM B 5	35.0	1040 45.5	4.4 1.0
6 85	RECR	F 627	PM B 10	37	1185 56	4.7 1.1
6 85	RECR	F 628	PM B 6	36	1075 43	4 0.8
6 85	RECR	F 629	PM B 4	34	760 25.5	3.4 1.1
6 85	RECR	F 631	PM B 9	40.5	1600 78	4.9 1.0
6 85	RECR	F 632	PM B 5	33	825 36.5	4.4 .
6 85	RECR	F 633	PM NB 4	36	905 46	5.1 .
6 85	RECR	F 635	PM NB 9	41	1580 59	3.7 .
6 85	RECR	F 637	PM B 9	43	1500 69	4.6 1.0
6 85	RECR	F 639	PM NB 10	40	740 53.5	7.2 .
6 85	RECR	F 640	PM NB 5	34.5	790 28.5	3.6 .
6 85	RECR	F 641	PM NB 5	33	820 42	5.1 .
6 85	RECR	F 643	PM NB 5	37.5	1080 52.5	4.9 .
6 85	RECR	F 645	PM NB 4	32.5	700 30.5	4.4 .
6 85	RECR	F 646	PM NB 3	30.5	555 25	4.5 .
6 85	RECR	F 647	AM B 4	34.5	840 30.5	3.6 1.0
6 85	RECR	F 649	AM B 10	40.5	1245 57	4.6 1.2
6 85	RECR	F 650	AM B 7	37.0	995 56.5	5.7 1.4
6 85	RECR	F 651	AM B 4	30	560 17	3.0 1.0
6 85	RECR	F 652	AM B 4	30.5	615 7	1.1 0.9
6 86	PRES	F 901	AM B 4	31.0	600 .	. .
6 86	PRES	F 902	AM B 4	31.0	625 .	. .
6 86	PRES	F 903	AM NB 4	31.5	645 .	. .
6 86	PRES	F 904	AM NB 5	32.0	670 .	. .
6 86	PRES	F 905	AM NB 5	28.5	530 52.0	9.8 .
6 86	PRES	F 906	AM NB 4	30.0	600 .	. .
6 86	PRES	F 907	AM NB 5	32.5	725 .	. .
6 86	PRES	F 908	AM NB 5	29.0	600 .	. .
6 86	PRES	F 909	AM B 10	38.0	1160 174	15 .
6 86	PRES	F 910	AM B 5	31.5	750 68.2	9.1 .

SITE	SEASON	NO	BLOOD	LENGTH	G WT	HSI
YR	SEX	TIME	AGE	WEIGHT	GSI	
6 86	PRES	M	911	AM NB 7	33.0 800 40.6 5.1	.
6 86	PRES	M	912	AM NB 5	29.5 545 22.4 4.1	.
6 86	PRES	M	913	AM NB 5	33.0 690 36.9 5.3	.
6 86	PRES	M	914	AM NB 7	30.5 670 33.7 5.0	.
6 86	PRES	M	915	AM NB 5	31.0 575 21.9 3.8	.
6 86	PRES	M	916	AM NB 5	31.0 610 18.0 3.0	.
6 86	PRES	M	917	AM NB 8	34.0 840 36.1 4.3	.
6 86	PRES	M	918	AM NB 5	30.5 560 17.3 3.1	.
6 86	PRES	F	919	AM NB 4	31.5 705 71.8 10.2	1.25
6 86	PRES	F	920	AM NB 5	34.5 990 112 11.3	1.42
6 86	PRES	F	924	PM B 10	43.0 1650 220.5 13.4	1.32
6 86	PRES	F	925	PM B 7	38.0 1075 135.7 12.6	1.61
6 86	PRES	F	926	PM B 8	35.5 1005 116.9 11.6	1.49
6 86	PRES	F	927	PM B 7	36.0 975 139.0 14.3	1.20
6 86	PRES	F	928	PM B 5	31.0 675 78.4 11.6	1.63
6 86	PRES	F	929	PM B 5	32.0 735 77.7 10.6	.
6 86	PRES	M	930	PM B 6	33.0 735 38.0 5.2	1.35
6 86	PRES	M	931	PM B 10	39.0 1205 56.2 3.0	1.33
6 86	PRES	M	932	PM B 5	31.0 660 24.3 3.7	1.26
6 86	PRES	M	933	PM B 5	32.5 780 30.9 3.9	1.38
6 86	PRES	M	934	PM B 5	31.0 570 25.1 4.4	1.09
6 86	PRES	F	935	AM B 4	32.5 695	.
6 86	PRES	F	936	AM B 5	34.0 795 92.8 11.6	.
6 86	PRES	F	937	AM B 7	38.0 1055 118 11.2	1.60
6 86	PRES	M	938	AM B 6	34.5 885 39.2 4.4	.
6 86	PRES	M	939	AM B 4	30.5 635 20.0 3.2	1.29
6 86	PRES	M	940	AM B 5	31.5 675 22.1 3.3	1.33
6 86	PRES	M	941	AM B 4	30.0 640 13.5 2.1	.
6 86	PRES	F	942	AM B 7	34.0 865 97.1 8.9	1.91
6 86	PRES	M	943	AM B 7	32.5 615 30.0 4.9	0.85
6 86	PRES	M	944	AM B 10	34.5 950 45.9 4.8	1.71
6 86	PRES	F	945	AM NB 7	37.0 1060 115.9 4.9	.
6 86	PRES	F	946	AM NB 10	44.5 2100 289.5 13.8	.
6 86	PRES	F	947	AM NB 10	45.5 2080 305.9 14.7	.
6 86	PRES	F	948	AM NB 7	35.5 1050 167.0 15.9	.
6 86	SPAW	F	949	PM B	.	.
6 86	SPAW	F	950	PM B	.	.
6 86	SPAW	F	951	PM B	.	.
6 86	SPAW	F	952	PM B	.	.
6 86	SPAW	F	953	PM B	.	.
6 86	SPAW	F	954	PM B	.	.
6 86	SPAW	M	955	PM B	.	.
6 86	SPAW	M	956	PM B	.	.
6 86	SPAW	M	957	PM B	.	.
6 86	SPAW	M	958	PM B	.	.
6 86	SPAW	M	959	PM B	.	.
6 86	SPAW	M	960	PM B	.	.

SITE	SEASON	NO	BLOOD	LENGTH	G WT	HSI
YR	SEX	TIME	AGE	WEIGHT	GSI	
6 86	SPAW	F	961	AM B	.	.
6 86	SPAW	F	962	AM B	.	.
6 86	SPAW	F	963	AM B	.	.
6 86	SPAW	F	964	AM B	.	.
6 86	SPAW	F	965	AM B	.	.
6 86	SPAW	F	966	AM B	.	.
6 86	SPAW	F	967	AM B	.	.
6 86	SPAW	M	968	AM B	.	.
6 86	SPAW	M	969	AM B	.	.
6 86	SPAW	M	970	AM B	.	.
6 86	SPAW	M	971	AM B	.	.
6 86	SPAW	M	972	AM B	.	.
6 86	SPAW	M	973	AM B	.	.
6 86	POST	F	974	AM B 5	37 1010 15.7 1.6	1.82
6 86	POST	F	975	AM B 10	40.5 1295 16.1 1.2	1.70
6 86	POST	M	976	AM B 10	39 1165 36.3 3.1	1.52
6 86	POST	F	984	AM NB 9	40 1135 13.5 1.2	1.67
6 86	POST	F	985	AM NB 7	36 885 16.5 1.9	1.30
6 86	POST	F	986	AM NB 9	41 1405 21 1.5	1.71
6 86	POST	F	990	PM NB 7	37 970 14.5 1.5	1.86
6 86	POST	F	991	PM NB 7	38 965 14 1.5	1.55
6 86	POST	F	992	PM NB 10	42 1630 24.5 1.5	1.58
6 86	POST	F	993	PM NB 5	32 760 .	1.38
6 86	POST	F	994	PM NB 10	42.5 1610 22 1.4	1.91
6 86	POST	F	9104	PM B 9	38 1205 .	.
6 86	POST	F	9105	PM B 5	34 735 .	.
6 86	POST	F	9106	PM B 6	35 820 .	.
6 86	POST	M	9113	PM B 10	38 935 16.4 1.8	.
6 86	RECR	M	501	PM NB 5	30.5 645 37 5.7	1.24
6 86	RECR	M	502	PM NB 4	32 675 39 5.8	1.19
6 86	RECR	F	510	PM NB 10	43.5 1420 90 6.3	1.48
6 86	RECR	M	511	PM NB 4	30.5 635 31 4.9	.
6 86	RECR	F	512	PM NB 4	28 520 .	.
6 86	RECR	F	513	PM NB 5	38 1185 49 4.1	1.27
6 86	RECR	M	514	PM NB 4	30.5 590 22 3.7	1.19
6 86	RECR	M	515	PM NB 5	31.5 685 40 5.8	1.17
6 86	RECR	F	516	PM NB 4	31 650 6 0.9	1.54
6 86	RECR	F	517	PM NB 4	32 715 39 5.5	1.26
6 86	RECR	F	518	PM NB 4	32 650 .	.
6 86	RECR	M	519	PM NB 6	33 945 54 5.7	.
6 86	RECR	F	520	PM NB 4	29.5 585 .	.
6 86	RECR	F	521	PM NB 5	34.5 825 .	.
6 86	RECR	F	522	PM NB 4	30.5 580 5 .	.
6 86	RECR	F	531	PM NB 5	37.5 1030 47 .	.
6 86	RECR	F	532	PM NB 4	31 635 .	.
6 86	RECR	F	533	PM NB 4	32 755 31 .	.
6 86	RECR	F	534	PM NB 4	32 715 31 .	.

Appendix C

FECUNDITY DATA

This appendix lists all of the data for female suckers collected and analyzed for fecundity estimates.

Legend: Site 1=MAN, 2=LMN, 3=LOK

Site	Length	Weight	Age	Egg Diam	Eggs per g	Fecundity
1	32	636	4	1.75	238.1	19900
1	31	750	7	1.70	250	27600
1	31	670	5	1.80	172.4	20300
1	30.5	713	4	1.65	243.9	28400
1	30.5	706	4	1.75	200	19800
1	34.5	755	9	1.85	192.9	20200
1	40	1170	10	2	166	31500
1	33	705	.	1.83	217.7	20600
1	33	785	6	1.95	176.9	22700
1	33.5	710	6	1.75	253.5	23800
1	34	805	8	1.85	190.9	21600
1	32	655	5	1.80	227.9	20900
1	32.5	705	8	1.88	183.2	20900
1	34.5	660	7	1.85	218.5	22600
1	35	775	6	1.88	205.6	26000
1	34	770	7	1.68	250.4	21500
1	33.5	830	8	1.85	214.8	25500
1	32.5	780	8	1.95	187.2	19700
1	30.5	680	7	1.83	223.6	17100
1	32	740	6	1.75	231.9	17700
1	32	670	6	1.7	262.5	19700
1	36	975	10	1.98	175.5	25700
1	32	730	8	1.95	204.8	19900
1	34.5	880	6	1.8	225	25200
1	37	980	10	2.05	171	30300
1	31.5	730	7	1.9	196.9	19400
2	32.5	835	6	1.65	259.5	23000
2	34	920	8	2	191.3	.
2	33.5	900	7	1.73	238.8	29500

Site	Length	Weight	Age	Egg Diam	Eggs per g	Fecundity
2	35	890	7	1.88	182.1	25900
2	32.5	825	7	1.85	216.5	25600
2	33	685	7	1.88	195.3	18400
2	34	735	8	1.85	192.7	16400
2	41	1475	10	2	155.2	32500
2	32.5	675	9	1.88	204	18900
2	34.5	830	8	1.83	211.7	24000
2	31.5	785	7	2	164	16800
2	32.5	640	7	1.85	196.9	13500
2	35.5	945	8	1.85	193	23100
2	31.5	650	7	1.78	215	19500
2	35	870	6	1.83	203.6	23200
2	33	740	7	1.68	259.3	21500
2	35	860	8	1.80	212	25600
2	32.5	780	8	1.88	200.1	17700
2	33	835	8	1.8	218.2	.
2	32	740	6	1.8	205	18500
2	35.5	950	7	1.78	226.6	24700
2	32.5	707	7	1.95	190.8	20500
2	33	730	6	1.85	200.7	19200
2	33	860	6	1.98	191	23000
3	32	925	5	1.8	200	25900
3	32.5	950	5	2	169.5	26100
3	28.5	530	5	1.73	245	17400
3	38	1160	10	1.95	170.5	33000
3	31.5	750	5	1.65	252.4	20600
3	31.5	705	4	1.83	236.3	19900
3	34.5	990	5	1.65	230.8	30500
3	43	1650	10	2.15	189.5	45700
3	38	1075	7	1.78	224.4	35700
3	35.5	1005	8	1.95	173.6	24100
3	36	975	7	1.93	190.7	32200
3	31	675	5	1.75	235.2	22800
3	32	735	5	1.78	239.6	23500
3	34	795	5	1.85	200.9	.
3	38	1055	7	1.95	198.5	28000
3	34	865	7	1.85	205.8	25400
3	39	1060	7	1.83	197.8	32200
3	44.5	2100	10	2.05	163.6	55900
3	45.5	2080	10	1.95	192.9	59100
3	35.5	1050	7	2	168.8	34800

Appendix D

BIOCHEMICAL DATA

The data collected during this study from blood, liver and muscle tissues of white suckers. Values are given for liver glycogen (GLY), blood protein (PRO), serum calcium (CA), total serum lipids (SERLIP), cholesterol (TOTCHOL), muscle lipids (MUSLIP), high-density lipid cholesterols (HDLCHOL), serum triglycerides (TRIGLYC) and alkali-labile serum phosphorus (PHOSP).

Site 1=MAN, 2=LOK Sex 1=Male 2=Female Season 1=PRES, 2=SPAW, 3=POST, 4=RECR.

SITE	SEAS	SEX	GLY	PRO	CA	SERLIP	MUSLIP	TOTCHOL	HDLCHOL	TRIGLYC	PHOSP
2	1	1	26.7	30.3	64.9	23.0	8.6	113.5	61.7	558	.
2	1	1	22.6	33.1	56.7	14.2	7.7	112.4	43.4	597	.
2	1	1	44.3	52.6	59.7	18.8	.	321.5	20.3	30	.
2	1	1	65.8	46.0	48.5	22.3	.	431.6	41.7	253	.
2	1	1	.	48.2	64.5	17.7	.	418.1	44.3	1011	.
2	1	1	.	36.8	51.6	24.9	.	419.3	.	.	.
2	2	1	5.4	35.5	61.2	29.4	8.1	277.6	61.0	1237	26.6
2	2	1	49.5	37.8	52.0	19.0	6.5	197.8	83.6	2317	3.0
2	2	1	35.4	33.5	43.2	33.8	7.2	257.4	37.4	975	26.8
2	2	1	76.8	39.6	52.6	24.9	7.1	265.3	50.8	1562	29.2
2	2	1	31.7	45.6	45.5	24.4	15.1	165.2	37.4	976	20.4
2	2	1	47	41.0	55.0	40.2	11.1	378.8	.	.	33.6
2	2	1	41.6	.	.	.	9.9	.	.	.	31.6
2	2	1	6.2	.	.	.	21.1
1	1	1	4.6	38.2	59.4	17.4	8.9	294.5	62.2	511	.
1	1	1	11.5	40.2	65.5	10.6	.	341.7	60.3	1260	.
1	1	1	49.2	37.0	55.0	32.4	.	345.1	72.9	30	.
1	1	1	.	41.3	41.7	27.5	.	350.7	55.3	565	.
1	1	1	.	40.1	52.0	21.8	.	384.4	62.4	1067	.
1	1	1	.	40.8	51.5	27.4	.	261.9	.	.	.
1	2	1	40.9	41.2	49.1	15.1	7.4	280	55.3	2540	2.0
1	2	1	47.6	40.5	61.6	8.7	6.8	230.4	36.9	2018	18.0
1	2	1	18.4	36.1	55.5	13.8	5.9	312.5	43.6	880	.

SITE SEAS			GLY	PRO	CA	SERLIP	TOTCHOL		TRIGLYC		
SEX						MUSLIP		HDLCHOL	PHOSP		
1	2	1	57.8	38.1	46.5	23.6	.	446.2	53.1	1198	28.6
1	2	1	47.3	36.1	47.9	32.1	.	310.2	60.3	1753	4.3
1	2	1	40.1	43.3	60.0	16.5	.	305.7	.	.	27.6
1	2	1	59.5
1	2	1	46.1
1	2	1	50.8
1	2	1	4.4
2	1	2	14.8	39.5	63.0	14.2	7.8	563.1	51.9	519	.
2	1	2	23.5	46.7	62.3	14.5	7	130.4	68.4	486	.
2	1	2	20.6	34.0	48.4	19.5	7.1	832.7	70.5	553	.
2	1	2	20.4	33.8	37.9	22.8	5.8	243.9	74.3	69	.
2	1	2	26.8	35.9	47.0	14.0	7.6	297.8	71	.	.
2	1	2	37.8	39.0	46.3	17.3	.	233.8	.	.	.
2	1	2	52.7
2	2	2	31.2	45.5	54.1	26.3	13.7	167.5	61.5	455	14.8
2	2	2	24.5	42.0	52.3	23.7	8.6	399	44.8	415	10.7
2	2	2	16.2	33.7	50.8	18.3	5.9	410.3	56	1198	9.2
2	2	2	43.7	41.1	52.1	27.7	10.9	285.5	67.2	674	1.5
2	2	2	33.9	48.0	54.8	19.9	6.2	418.1	45.5	525	2.6
2	2	2	34.8	36.1	52.0	24.0	.	392.3	.	.	13.3
2	2	2	3.6
2	2	2	8.7
1	1	2	21	38.9	54.5	20.2	6.1	297.9	63.6	346	.
1	1	2	2.9	42.9	45.6	18.7	7.8	256.3	30.7	1163	.
1	1	2	19.3	34.1	55.6	12.5	5.5	258.5	74.3	901	.
1	1	2	.	38.0	45.4	22.8	7.7	405.8	63.9	433	.
1	1	2	.	34.0	53.5	15.2	7	460.8	63.1	249	.
1	1	2	.	37.6	.	12.7	.	350.7	46	.	.
1	2	2	17.4	40.5	56.6	28.5	3.3	378.8	52.7	604	7.6
1	2	2	11	39.6	47.3	17.7	4.7	456.3	82.7	817	7.5
1	2	2	56.4	58.5	48.6	23.9	7.3	431.6	51	990	12.5
1	2	2	9.5	38.0	57.0	33.6	8.1	432.7	83.2	2870	2.8
1	2	2	23.5	39.4	54.1	12.9	6.9	401.3	.	625	14.0
1	2	2	7.2	37.5	52.5	15.9	7	383.3	.	.	8.9
1	2	2	8.5	.	.	.	5.8	.	.	.	10.7
1	2	2	8.7	.	.	.	7.5	.	.	.	17.6
1	2	2	41.7
2	1	3	77.7	39.2	44.1	9.4	.	341.6	58.1	1254	.
2	1	3	65.9	35.0	53.9	22.5	.	139.4	19.3	821	.
2	1	3	49.9	48.0	56.3	27.5	.	367.5	55.8	2207	.
2	1	3	3.9	48.2	63.7	16.0	.	360.8	60.1	1106	.
2	1	3	11.5	37.0	52.2	25.5	.	383.3	53.9	1529	.
2	1	3	49.5	46.4	65.8	36.9	.	285.5	.	.	.
2	1	3	60
2	2	3	5.2	35.8	44.6	13.5	.	157.4	39.8	1577	33.2
2	2	3	26	37.1	42.3	12.3	.	164.1	41.7	162	13.8
2	2	3	41.2	40.2	39.5	24.4	.	392.3	42.9	968	22.3

SITE	SEAS				SERLIP	TOTCHOL	TRIGLYC		
SEX	GLY	PRO	CA		MUSLIP	HDLCHOL	PHOSP		
2 2 3	33.9	45.2	49.6	22.5	.	241.7	43.6	374	3.3
2 2 3	46.4	39.4	48.0	23.2	.	267.5	62	1121	8.6
2 2 3	52.7	33.7	57.7	23.6	.	311.3	.	.	20.0
2 2 3	13.2
1 1 3	66.5	37.0	40.4	22.9	.	82.0	57.7	548	.
1 1 3	67.8	28.6	52.0	13.3	.	415.6	40	965	.
1 1 3	87.1	41.9	46.2	39.2	.	421.5	64.8	1211	.
1 1 3	68.9	38.5	37.1	19.8	.	236	36.2	939	.
1 1 3	76.7	37.1	52.6	.	.	392.3	56.2	968	.
1 1 3	.	44.7	55.3	.	.	340.6	.	.	.
1 2 3	25.6	42.7	47.0	12.5	.	305.6	36.5	156	7.6
1 2 3	33.1	43.7	46.8	10.0	.	158.5	43.8	847	29.3
1 2 3	35.8	38.7	38.8	29.1	.	335	40	520	8.2
1 2 3	29.1	36.2	43.5	15.7	.	341.7	55	514	20.0
1 2 3	50.5	37.5	30.0	.	.	284.4	57.7	704	7.6
1 2 3	12	59.1	51.1	.	.	342.8	.	.	22.2
1 2 3	29.1
1 2 3	8.7
2 1 4	.	40.1	65.5	27.9	.	372	67.7	379	.
2 1 4	.	41.6	58.8	18.6	.	346.2	73.4	1250	.
2 1 4	.	65.1	68.4	27.0	.	365.3	65.1	1178	.
2 1 4	.	36.6	52.5	23.7	.	391.2	53.4	410	.
2 1 4	.	38.2	51.1	26.1	.	366.4	55.3	556	.
2 1 4	.	41.5	53.8	41.6	.	291.1	.	.	.
2 2 4	.	31.2	58.4	18.9	.	124.8	63.9	162	10.4
2 2 4	.	32.8	51.4	9.3	.	324.8	42.9	121	5.1
2 2 4	.	52.5	51.5	13.1	.	232.7	28.6	315	4.4
2 2 4	.	52.1	58.5	18.4	.	167.5	50.5	141	27.1
2 2 4	.	38.4	49.0	18.8	.	203.4	69.6	529	3.6
2 2 4	.	37.7	49.0	15.3	.	248.4	.	.	14.1
2 2 4	11.0
1 1 4	.	40.5	61.9	21.9	.	268.6	69.1	230	.
1 1 4	.	30.0	52.0	37.4	.	417	71.5	509	.
1 1 4	.	44.8	55.3	32.3	.	402.4	70.3	453	.
1 1 4	.	49.8	58.0	30.9	.	372	69.1	837	.
1 1 4	.	38.4	53.8	36.4	.	485.6	79.6	243	.
1 1 4	.	43.1	55.0	36.4	.	342.8	.	.	.
1 2 4	.	40.7	51.9	17.0	.	347.3	59.1	177	7.9
1 2 4	.	49.9	52.7	12.9	.	327.1	68.2	1772	2.0
1 2 4	.	32.6	58.6	20.0	.	213.6	54.1	266	11.8
1 2 4	.	39.3	47.5	30.8	.	347.3	61.7	453	2.3
1 2 4	.	41.2	65.6	21.6	.	285.5	53.9	20	1.0
1 2 4	.	42.5	48.1	19.1	.	327.1	.	.	3.3

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